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## COMMUNICATIONS

### 5-hydroxytryptamine (5-HT) dependent myoclonus in the guinea-pig may provide evidence for multiple cerebral 5-HT receptors

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Myoclonus induced by administration of L-5-hydroxytryptophan (5-HTP) to guinea-pigs appears to be due to stimulation of cerebral 5-hydroxytryptamine (5-HT) receptors (Klawans, Goetz & Weiner, 1973; Chadwick, Hallett, Jenner & Marsden, 1978), and has been suggested as an animal behavioural model for detecting drugs modulating central 5-HT function (Volkman, Lorens, Kindel & Ginos, 1978). We have investigated the capacity of a range of 5-HT antagonists and re-uptake inhibitors to modulate 5-HTP-induced myoclonus, and have determined the ability of synthetic 5-HT agonists to induce this behaviour.

Administration of 5-HTP (20–90 mg/kg, s.c.) to female guinea-pigs pretreated with the peripheral decarboxylase inhibitor, carbidopa (α-methyldopahydrazine; 25 mg/kg, i.p.; 60 mins previously) induced dose-dependent myoclonus. Similarly, tryptophan (50–200 mg/kg, s.c.) administered to animals pretreated with pargyline (75 mg/kg, i.p.; 60 mins previously) also produced myoclonus. In contrast, the synthetic 5-HT agonists quipazine (1.0–160 mg/kg, i.p.), MK212 (6-chloro-2-[1-piperazinyl]pyrazine; 1.0–80 mg/kg, i.p.) and 1-(m-trifluoromethylphenyl)-piperazine (1.0–160 mg/kg) evoked occasional myoclonic jerking only at the highest doses used.

Using 5-HTP (75 mg/kg, s.c., plus carbidopa, 25 mg/kg, i.p.) as a dose producing rhythmic myoclonus, a range of 5-HT receptor antagonists (injected 60 mins prior to, or at, the time of 5-HTP administration) were tested for their ability to inhibit myoclonic jerking. Methergoline (1.0–20 mg/kg, $P < 0.05$) and cyproheptadine (5.0–20 mg/kg, $P < 0.05$) were potent, dose-dependent inhibitors when given prior to or concurrent with 5-HTP administration. In contrast, effective inhibition was only produced by high doses of

mianserin (10–20 mg/kg, $P < 0.05$), methysergide and BW501C-67 (α-anilino-N-2-m-methoxyphenoxypropyl-acetamidine, both 20 mg/kg, $P < 0.05$).

The effect of a number of 5-HT re-uptake blockers (injected 10 min prior to 5-HTP) was investigated using a threshold dose of 5-HTP (20 mg/kg) which rarely induced myoclonus. The behavioural effects of 5-HTP (20 mg/kg) were markedly potentiated by chlorimipramine (2.5–20 mg/kg; $P < 0.05$), paroxetine and Org 6582 (5.0–20 mg/kg, $P < 0.05$), while femoxatine (10 or 20 mg/kg, $P < 0.05$) was only weakly active and fluoxetine and desmethylimipramine both had little effect in doses up to 20 mg/kg.

In conclusion, although 5-HTP-induced myoclonus in the guinea-pig does appear dependent on cerebral 5-HT mechanisms, putative 5-HT agonists, antagonists and re-uptake blockers do not have uniform effects on this behaviour. The differences observed cannot be attributed entirely to poor penetration of the ineffective drugs into the brain, to a lack of efficacy at central 5-HT receptors or to actions on other brain neuronal pathways. So this data may provide evidence for multiplicity of cerebral 5-HT receptors.

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## The effects of a selective uptake blocker on 5-HT turnover in the CNS

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Although many antidepressant drugs are known to be selective inhibitors of either 5-hydroxytryptamine (5-HT) or noradrenaline (NA) uptake, acutely, relatively little is known about adaptive changes in monoamine metabolism secondary to uptake inhibition following the chronic administration of these agents. Such changes may be of particular relevance when considered in relation to the findings that therapeutic efficacy is not immediately apparent following the commencement of chemotherapy with these compounds (Kielholz & Poeldinger, 1968; Alpers & Himwich, 1972).

The amine uptake studies of Sugden (1974) on Indoramin (Wy 21901) have been extended to the related compound Wy 25093 1-[1-([Indol-3-yl]methyl)piperid-4-yl]-3-benzoylurea. Wy 25093 was found to be a selective and potent 5-HT uptake inhibitor, *in vitro*, using cerebral cortex slices prepared from rat and monkey (*Erythrocebus patas patas*). Selectivity of monoamine uptake inhibition relative to NA and dopamine (DA) was demonstrated *in vitro* and for NA *in vivo*. Behavioural studies were also consistent with an action of Wy 25093 on the 5-HT uptake process. This was indicated by the antagonism of *p*-chloroamphetamine-induced hyperactivity and the potentiation of the behavioural effects of 5-hydroxytryptophan in rodents, in accordance with the methods described by Buus Lassen (1978).

We describe here the effects of long term administration of Wy 25093 on the 5-HT and 5-hydroxyindole-3-acetic acid (5-HIAA) levels in rat and monkey brain. 5-HT and 5-HIAA were assayed fluorimetrically by the method of Curzon & Green (1970).

The acute treatment of male rats (Sprague–Dawley, 180–250 g) with Wy 25093, caused a significant decrease in whole brain 5-HIAA concentration without affecting that of 5-HT. In contrast, rats treated with the compound for 22 days exhibited a significant decrease in levels of both 5-HT and 5-HIAA (Table 1). A similar effect was observed in cortical tissue from Patas monkeys, after daily dosing with Wy 25093 for 6 months.

Inhibition of 5-HT re-uptake, acutely, leads to a decrease in brain 5-HIAA levels, as has been observed with fluoxetine (Fuller, Perry & Molloy, 1974). This phenomenon is considered to result from a decreased rate of turnover of the amine as a result of feedback inhibition of synthesis, mediated by the postsynaptic receptor (Meek & Werdinius, 1970; Schubert, Nyback & Sedvall, 1970). In the chronically-treated animals, there was in addition the significant decrease in 5-HT levels. Although this reduction in 5-HT levels would

**Table 1** Levels of 5-HT and 5-HIAA after acute and chronic administration of Wy 25093

| *Species* | *Dose (mg/kg)* | *Days* | *5-HT ng/g brain†* | *5-HIAA (wet weight)* |
|---|---|---|---|---|
| Rat | Control | 1 | 619 ± 22 | 302 ± 5 |
| | 50 | 1 | 636 ± 11 | 232 ± 11* |
| Rat | Control | 22 | 804 ± 32 | 372 ± 12 |
| | 50 | 22 | 572 ± 20*** | 298 ± 19** |
| Monkey | Control | 183 | 157 ± 15 | 228 ± 30 |
| | 7.5 | 183 | 133 ± 20 | 117 ± 13** |
| | 15.0 | 183 | 84 ± 3*** | 132 ± 9* |
| | 30.0 | 183 | 97 ± 6** | 114 ± 7** |

All doses were administered by gavage, using hydroxypropylmethyl cellulose as the vehicle. Controls received vehicle alone. † whole brain—rat, cortex—monkey. Results are the mean ± s.e. mean of 5–8 experiments. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

appear to counteract any effect of uptake inhibition, it may indicate an adaptive change to increased levels of 5-HT in the synaptic cleft. A similar adaptation, but to a reduction of 5-HT in the synaptic cleft has been described as receptor supersensitivity (Steigrad, Tobler, Waser & Barbely, 1978). Both changes acting in apposition emphasise the importance of homeostatic mechanisms in mammals.

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## Use of amphetamine at high dosage in the study of DA-5HT interactions: effects of neuroleptics


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The well-known dopamine (DA)-dependent response to (+)-amphetamine (increased forward locomotion, rearing, head bobbing, gnawing, etc.) is altered at high doses (25 mg/kg, i.p.). Head bobbing remains prominent but forward locomotion decreases, other effects largely disappear and backward walking and circling occur (Taylor, Goudie, Mortimore & Wheeler, 1974). These latter behaviours appear to be mediated by concurrent DA and 5-hydroxytryptamine (5-HT) release (Lees, Fernando & Curzon, 1979; Curzon, Fernando & Lees, 1979). 'Classical' 5-HT-dependent behaviours (reciprocal forepaw treading, hind-limb

abduction, 'wet-dog' shakes, etc.) of controversial DA dependence (Heal, Green, Boullin & Grahame-Smith, 1976; Sloviter, Drust & Connor, 1978) also occur. We have investigated the roles of DA and 5-HT in the above behaviours by studying their inhibition by various neuroleptics.

Male Sprague–Dawley rats (180–200 g) housed as previous described (Curzon *et al.*, 1979) were given neuroleptics i.p. suspended in 0.5% BRIJ (polyoxyethylene lauryl ether, BDH) at 2.5 ml/kg body weight. Controls were given vehicle alone. 1 h later, all rats were given 25 mg/kg i.p. (as base) of (+)-amphetamine sulphate. Behaviours were scored 'blind' from 5 to 60 min after amphetamine injection (between 1000–2000 h or between 1500–1600 h) in 'all or none' fashion (Curzon *et al.*, 1979). Each neuroleptic was given at a range of doses and $ID_{50}$ calculated for inhibition of backward walking. Pimozide, sulpiride, haloperidol and metoclopramide inhibited backward walking at doses affecting neither head bobbing nor 'classical' 5-HT behaviour. Other drugs which inhibited head bobbing (bromoperidol, α-flupenthixol), 'classical' 5-HT behaviours (thioridazine) or both types of behaviour (clozapine) also inhibited backward walking and circling at the same dose in agreement with previous evidence that blocking either DA or 5-HT receptors suppresses backward walking and circling. Requirement of 5-HT as well as DA for backward walking is also indicated by ratios of $ID_{50}$ (apomorphine stereotypy)/$ID_{50}$ (backward walking) calculated using apomorphine data of Leysen, Niemegeers, Tollenaere & Laduron (1978). Ratios are in the order: pimozide << sulpiride < haloperidol < metoclopramide < α-flupenthixol < thioridazine < clozapine. This order is almost identical with that found by Leysen *et al.* for ratios of $ID_{50}$ (apomorphine stereotypy)/$ID_{50}$ (tryptamine seizures) which presumably increase as antagonism at 5-HT receptors increases relative to that at DA receptors.

The ratios also suggest that 'classical' 5-HT-dependent behaviour depends less on DA than does backward walking as only two of the eight drugs tested inhibited the former behaviour at doses which inhibited the latter (thioridazine and clozapine). These drugs had the two highest $ID_{50}$ ratios and may therefore have inhibited 'classical' 5-HT behaviour by blocking 5-HT receptors.

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## Role of 5-HT in shock-induced analgesia


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Acute stress, e.g. exposure to foot shock for as little as 30 s markedly increases thresholds of response to subsequent noxious stimulation (Madden, Akil, Patrick & Barchas, 1977; Hayes, Bennett, Newlon & Mayer, 1978). As evidence implicates 5-HT in the control of nociception (Messing & Lytle, 1977) we have examined the effects of serotonergic agents on the analgesia induced by shock.

Responses to noxious heat following acute inescapable electric shock to the feet for 30 s (1 mA, 6 pulses of 5 s duration, less than 1 s between pulses) were monitored in male Sprague–Dawley rats (234 ± 12 g) using the hot plate (plate at 55°C) or tail immersion (water bath at 51°C) technique and at least nine rats/group. In both tests, latencies to lick the paws or withdraw the tail were measured immediately before ($T_1$ s) and immediately after ($T_2$ s) exposure to

shock. Non-shocked controls were returned to the home cage for 30 s between determination of $T_1$ and $T_2$. The effect of electric shock on response was expressed as percentage analgesia score (PAS) = $T_2/T_1 \times 100$.

Using the hot plate test, footshock significantly increased PAS from 106 ± 9% to 219 ± 28% (mean of nine separate experiments ± s.d.). Smaller but significant increases were found using the tail immersion test. Administration of the 5-HT synthesis inhibitor *p*-chlorophenylalanine (150 mg/kg, daily for 3 days) decreased forebrain 5-HT concentration by 81% and significantly increased both hot plate and tail immersion PAS values 24 h after the last injection. Conversely, the analgesic effect of footshock (hot plate method only) was significantly attenuated by the 5-HT releasers *p*-chloroamphetamine and fenfluramine (1.5 mg/kg and 2.5 mg/kg respectively, 30 min before testing); by the inhibitor of 5-HT re-uptake fluoxetine (10 mg/kg, 2 h before testing) and by the 5-HT agonist 5-methoxy-N,N-dimethyltryptamine (2 mg/kg, 20 min before testing). Another agonist, 1-(m-trifluoromethylphenyl) piperazine (10 mg/kg, 2 h before testing) and three antagonists, metergoline (1 and 5 mg/kg, 2 h before testing), methysergide (2 mg/kg, 2 h before testing) and cyproheptadine (10 mg/kg, 20 min before testing) were all without effect. These results, while they strongly indicate a relationship between 5-HT availability and analgesia, provide scant evidence for the involvement of conventional 5-HT receptors.

Results in general strongly suggest that some 5-HT neurons inhibit the analgesic response to shock. The mechanism may be of physiological significance as in some experiments (albeit not all) significant negative correlations between PAS (hot plate method) and forebrain 5-HT concentration were found in non-drug treated animals. The inverse relationships found differ from other evidence of a positive association between 5-HT and threshold of response to noxious stimulation (Messing & Lytle, 1977). One possibility is that 5-HT opposes shock-induced analgesia by reducing awareness of electroshock.

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## Presynaptic dopamine receptors modulate electrical stimulation but not amphetamine-evoked release of $^3$H-dopamine from the rabbit caudate nucleus

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Presynaptic modulation of neurotransmission operates only when transmitter release is triggered by a calcium dependent mechanism. Thus, presynaptic regulation through α-adrenoceptors has been demonstrated for release of noradrenaline elicited by either high potassium or field stimulation (Langer, 1974, 1977; Starke, 1977) but not for tyramine which releases monoamines through a calcium independent mechanism (Starke and Montel, 1974; Pelayo, Dubocovich & Langer, 1978). Modulation by presynaptic dopamine (DA) receptors of the electrically stimulated release of DA has been reported to occur in the rabbit caudate nucleus (Starke, Reimann, Zumstein & Hertting, 1978). Since amphetamine is also able to release DA we have compared the presynaptic modulation of the release of $^3$H-DA elicited by electrical stimulation with that elicited by amphetamine. The effects of apomorphine and haloperidol on the release of $^3$H-DA induced by field stimulation and amphetamine were studied in slices of the rabbit caudate nucleus prelabelled with $^3$H-DA and superfused as described by Starke *et al.*, (1978). Two periods ($S_1$, $S_2$) of either field stimulation (3 Hz, 2 ms, during 2 min with a current of 24 mA) or amphetamine (10 μM, 2 min) were applied with an interval of 44 min and drugs were added 20 min before $S_2$. The percentage of total tissue radioactivity released by field stimulation during $S_1$ was 3.80 ± 0.18% ($n$ = 23) and for $S_2$: 3.42 ± 0.18% ($n$ = 23). The ratio $S_2/S_1$ was 0.90 ± 0.02 ($n$ = 23). The release of $^3$H-DA induced by electrical stimulation was found to be entirely cal-

cium dependent ($S_2/S_1$: 0.11 ± 0.10, $n = 6$, $P < 0.001$), when $S_2$ was carried out in $Ca^{2+}$ free Krebs' solution.

Under the same experimental conditions amphetamine (10 μM) released during $S_1$: 4.07 ± 0.19 ($n = 20$) percent of total tissue radioactivity, and during $S_2$ 3.41 ± 0.21% ($n = 20$). The ratio $S_2/S_1$ was 0.83 ± 0.03 ($n = 20$). The release of $^3$H-DA elicited by amphetamine was found to be entirely calcium independent ($S_2/S_1$: 0.81 ± 0.06, $n = 8$), when $S_2$ was carried out in $Ca^{2+}$ free Krebs' solution.

Apomorphine produced a concentration dependent decrease in the release of $^3$H-DA evoked by field stimulation. In the presence of apomorphine ($10^{-7}$ M), the ratio $S_2/S_1$ was 0.22 ± 0.06 ($n = 8$, $P < 0.001$) and for apomorphine ($10^{-6}$ M) the ratio $S_2/S_1$ was 0.10 ± 0.01 ($n = 6$, $P < 0.001$).

Under the same experimental conditions apomorphine was practically ineffective in reducing the amphetamine evoked release, the ratio $S_2/S_1$ was 0.65 ± 0.06, ($n = 12$, $P < 0.02$) for apomorphine $10^{-7}$ M while at $10^{-6}$ M, no inhibition could be observed ($S_2/S_1$; 0.82 ± 0.02, $n = 8$).

Haloperidol produced a concentration dependent increase of $^3$H-DA release evoked by field stimulation. In the presence of haloperidol ($10^{-9}$ M) the ratio $S_2/S_1$ was 1.08 ± 0.03, ($n = 7$, $P < 0.001$) and at $10^{-8}$ M the ratio $S_2/S_1$ was 1.32 ± 0.01, ($n = 6$, $P < 0.001$).

The amphetamine induced release of $^3$H-DA in the presence of haloperidol ($10^{-9}$ M) was not significantly different from control $S_2/S_1$: 0.93 ± 0.07, ($n = 7$). At $10^{-8}$ M haloperidol a small but statistically significant increase was observed, the ratio $S_2/S_1$ was 0.94 ± 0.04, ($n = 7$, $P < 0.05$). At $10^{-7}$ M haloperidol the ratio $S_2/S_1$ was 1.03 ± 0.02, ($n = 8$, $P < 0.02$), however, the increased release of $^3$H-DA evoked by amphetamine was accompanied by an increase in the spontaneous outflow of radioactivity by this concentration of haloperidol.

Tyramine displaces $^3$H-DA from vesicular storage sites through a calcium-independent mechanism. We also examined in similar experiments the effects of apomorphine and haloperidol on the release of $^3$H-DA by tyramine. In the controls exposure to tyramine (5 μM) for 2 min produced a release in $S_1$ of 4.40 ± 0.28% ($n = 9$) of total tissue radioactivity and 3.50 ± 0.28 for $S_2$. The ratio $S_2/S_1$ was 0.79 ± 0.02, ($n = 9$). The release of $^3$H-DA induced by tyramine was not modified by haloperidol at either $10^{-8}$ M or $10^{-7}$ M.

It can be concluded that the stimulated release of $^3$H-DA from the rabbit caudate induced by a calcium dependent mechanism is modulated by presynaptic inhibitory DA receptors, whereas the calcium independent evoked release of $^3$H-DA by amphetamine or tyramine is not modulated through inhibitory presynaptic DA autoreceptors.

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## Effects of partial, unilateral nigro-striatal lesions on striatal histofluorescence and behavioural indices of dopamine transmission


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Most investigators of nigro-striatal function who have used the catecholamine neurotoxin 6-hydroxydopamine (6-OHDA) have attempted to destroy as much of the nigrostriatal dopamine (DA) projection as possible. In view of the probable topographic nature of this pathway (Fallon & Moore 1978) we have determined the effects of a small standard dose of 6-OHDA injected at different locations within the ventral tegmentum on striatal histofluorescence and changes in locomotor activity and rotational behaviour induced by amphetamine and apomorphine.

Each of 30 female hooded rats was pre-treated with

desmethylimipramine (25 mg/kg). Of these animals 24 received stereotaxic injections of 6-OHDA-hydrobromide (2 μg free base in 0.5 μl of 1% ascorbate-saline solution at a rate of 0.1 μl/min) while six received an equivalent volume of the ascorbate vehicle. The co-ordinates of the injections were AP-2.5, HV-7.8, while the lateral co-ordinate was varied systematically, L 0.0–3.0 (Pelligrino & Cushman 1967). An additional six animals served as unoperated controls. On days 11, 12 and 13 after surgery, behavioural measures of locomotor activity and rotation were obtained following the counterbalanced administration of apomorphine (2 mg/kg), amphetamine (1 mg/kg) and saline (0.9%). On day 14 the striatal tissue of each animal was processed for glyoxylic-acid induced fluorescence of DA (de la Torre & Surgeon 1976, Redgrave 1978); the location of the tegmental injection was also determined.

The statistically significant results were as follows: (i) Intensity measurements of striatal fluorescence indicated a greater DA depletion in animals treated with 6-OHDA relative to the ascorbate ($P < 0.05$) and untreated animals ($P < 0.01$). (ii) Depletion of DA fluorescence was most when the lateral injection co-ordinate was 1.3 mm ($P < 0.025$). (iii) A significant medial/medial, lateral/lateral relationship was established between the region of maximal DA depletion within the striatum and lateral injection co-ordinate ($P < 0.001$). (iv) Ipsilateral rotation induced by amphetamine was linearly related to the extent of overall depletion within striatum ($r = 0.52$, $P < 0.025$). A similar relationship was established for contralateral rotation induced by apomorphine ($r = 0.55$, $P < 0.025$). (v) Turning induced by apomorphine was positively related to the HV injection co-ordinate with substantia nigra ($r = 0.56$, $P < 0.025$). (vi) Amphetamine potentiation of locomotor activity was also positively related to the HV injection co-ordinate ($r = 0.86$, $P < 0.001$), while a suppression of locomotor activity by apomorphine was inversely related to the HV co-ordinate ($P < 0.05$).

It is concluded from these data, firstly that both the extent and regional distribution of DA depletion within the striatum is dependent upon the precise co-ordinates at which 6-OHDA is injected into substantia nigra. Secondly, it appears that the amount of rotation induced by DA agonists is positively related to the extent of imbalance in DA between striata on each side of the brain. Finally, rotation induced by apomorphine and activity responses caused by both DA agonists seem to be particularly affected by the dorso-ventral placement of 6-OHDA within the ventral tegmentum.

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## [$^3$H]-Spiperone labels dopamine receptors in homogenates of bovine retina


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[$^3$H]-Spiperone, a potent neuroleptic drug, has been used to label not only dopamine receptors, but also serotonin receptors in several regions of the brain (Fields, Reisine & Yamamura, 1977; Leysen, Niemegeers, Tollanaere & Laduron, 1978). Dopamine is also known to be an important neurotransmitter in the retina (Ehinger, 1976) and in the present study [$^3$H]-spiperone was used to identify dopamine receptors in homogenates of bovine retina.

Specific binding of [$^3$H]-spiperone, as defined by (+)-butaclamol (1 μM), was saturable and Scatchard analysis of the saturation data indicated a single population of binding sites with a dissociation constant ($K_d$) of 0.38 ± 0.04 nM and a maximum number of binding sites ($B_{max}$) of 64.8 ± 12.0 fmol/mg protein. Using [$^3$H]-spiperone (0.37 nM) and a tissue concentration of approximately 1 mg protein/ml, specific binding represented 80–90% of the total bound radioactivity.

In drug inhibition experiments, spiperone and the ergot derivative lisuride were the most potent displacers with $IC_{50}$ values of 0.74 nM and 2.7 nM re-

**Table 1** Inhibition of [$^3$H]-spiperone binding to homogenates of bovine retina

| *Drug* | *$IC_{50}$ (M)* |
|---|---|
| Spiperone | $7.4 \times 10^{-10}$ |
| Lisuride | $2.7 \times 10^{-9}$ |
| Haloperidol | $1.0 \times 10^{-8}$ |
| Apomorphine | $2.8 \times 10^{-8}$ |
| α-Flupenthixol | $4.2 \times 10^{-8}$ |
| Fluphenazine | $6.0 \times 10^{-8}$ |
| (+)-Butaclamol | $6.8 \times 10^{-8}$ |
| Dopamine | $1.0 \times 10^{-6}$ |
| ADTN† | $3.2 \times 10^{-6}$ |
| Cinanserin, Phentolamine, Propranolol, (−)-Butaclamol, 5-Hydroxytryptamine | $>1 \times 10^{-5}$ |

Six concentrations of each drug, in the range $10^{-11}$–$10^{-4}$ M were examined for their ability to inhibit [$^3$H]-spiperone binding (0.37 nM). Each drug concentration was performed in triplicate on 2–3 separate occasions. $IC_{50}$ values, determined by log-probit analysis of resultant inhibition curves, represent the concentration of drug required to produce 50% inhibition of specific binding as defined by $10^{-6}$ M (+)-butaclamol.
† 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene.

spectively (see Table 1). Various dopamine antagonists including haloperidol, (+)-butaclamol, fluphenazine and α-flupenthixol also inhibited $^3$H-spiperone binding with $IC_{50}$ values in the range 10–68 nM (Table 1). The dopamine agonist apomorphine, with an $IC_{50}$ value of 28 nM, was some 35 times more potent than dopamine itself. In marked contrast, serotonin and the serotonin antagonist cinanserin, together with the α- and β-adrenoreceptor blocking agents phentolamine and propranolol respectively, were essentially inactive, with $IC_{50}$ values greater than 10 μM.

In summary, the pharmacological specificity of $^3$H-spiperone binding in bovine retina suggests that $^3$H-spiperone binds to dopamine rather than to serotonin receptors. Furthermore, these data suggest that $^3$H-spiperone labels dopamine receptors of the D2 type in bovine retina, in contrast to our previous suggestion that D2 receptors appear to be absent from guinea-pig and carp retinae (Watling, Dowling & Iversen, 1979).

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## Dopaminergic modulation of striatal [$^3$H]-glutamic acid release

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The neostriatum receives a major input from cerebral cortex which may be, at least partly, glutamatergic in nature (McGeer, McGeer & Hattori, 1979). In addition to reductions in striatal glutamic acid levels, uptake and release, cortical ablation results in a partial loss of striatal [$^3$H]-haloperidol binding sites (Schwarz, Creese, Coyle & Snyder, 1978).

The present experiments were carried out to determine whether these implied DA receptors on glutamatergic terminals could functionally modify the transmitter release and further to investigate whether they could be classified as D1 or D2 type receptors (Kebabian & Calne, 1979).

The method of investigation of transmitter release has been described in detail previously (Mitchell & Martin, 1978). The technique employs a continuous superfusion which effectively eliminates re-uptake of released transmitter. Briefly, 0.1 mm prisms of striatal tissue were suspended in physiological medium and incubated with 1-[2,3-$^3$H]-glutamic acid at a concentration of 0.3 μM to allow high affinity uptake (Mulder & Snyder, 1974). Tissue was then loaded onto filters in chambers kept at 37°C and continuously superfused with medium. Fractions of the perfusate were collected and radioactivity determined by liquid scintillation counting. After several minutes when a steady rate of basal efflux was reached, more medium

was added. This was either normal, or with 40 mM $K^+$ (a submaximal pulse) and containing the compounds under study. The identity of the radioactivity in the perfusate was investigated by thin-layer chromatography, and during basal efflux some 69% of the released radioactivity migrated with the $R_F$ of authentic glutamate, whilst during the 40 mM $K^+$ pulse this figure rose to 90%. The stimulus-induced release was reduced by 65% when $Mg^{2+}$ was substituted from $Ca^{2+}$ in the medium.

At concentrations above 50 μM, DA caused a significant inhibition of $K^+$-induced glutamate release, whilst (−)-NA and 5-HT even at 1000 μM had no effect. This effect was mimicked by several DA agonists which showed an order of potency of apomorphine > ADTN > DA and also by the ergot derivatives bromocriptine and lergotrile which were of similar potency to apomorphine. These compounds inhibited $K^+$-induced release without any modification of basal efflux. The inhibition of $K^+$-induced release caused by 50 μM apomorphine (−49%) was blocked completely by 1 μM spiroperidol and partly by 20 μM haloperidol. The inhibitory effect of apomorphine and its blockade by spiroperidol could also be seen using synaptosomal preparations. The 'D2-receptor selective' DA antagonist sulpiride also completely blocked the apomorphine effect at 500 μM. None of these antagonists affected control $K^+$-induced release or basal efflux at these concentrations. The effect of DA agonists could not be mimicked by dibutyryl cAMP at a concentration of 1200 μM, (effective at modifying nigral GABA release, which appears to be modulated by a D1 type DA receptor, (Reubi, Iversen & Jessell, 1977)).

The potent effects of apomorphine, bromocriptine and lergotrile (which may be true agonists only at D2 and not D1 receptors (Kebabian & Calne, 1979)), the blockade of the effect by more or less selective D2 antagonists and the failure of dibutyryl cAMP to mimick the response all suggest that a D2-like DA receptor located on the presynaptic terminals mediates the inhibition of striatal glutamate release seen here.

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## Dopamine agonist and antagonist action after mesolimbic denervation

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Behavioural and biochemical studies have indicated that dopamine and/or neuroleptic sites may quantitatively change subsequent to denervation (Marshall & Ungerstedt, 1977; Cross, Longden, Owen, Poulter & Waddington, 1978; Costall, Fortune, Naylor & Nohria, 1979). The present studies were designed to assess whether such changes are relevant to dopamine agonist and antagonist action following mesolimbic denervation in the mouse. Climbing and circling were taken as behavioural indices (Costall, Naylor & Nohria, 1978; Costall et al., 1979) and denervation was caused by intra-accumbens 6-hydroxydopamine (6-OHDA). The 6-OHDA (2 μg, following pretreatment with 5.0 mg/kg i.p. tranylcypromine, 2 h, and 25 mg/kg i.p. desmethylimipramine, 1 h) was injected 2.3 mm anterior to bregma, ±1.0 mm lateral and 3.7 mm below the skull surface using male albino mice (B.K.W., 35–40 g) and standard stereotaxic techniques (Costall et al., 1979). Assessments on the 14th postoperative day showed 6-OHDA to cause a 70% depletion of mesolimbic dopamine but no significant change in mesolimbic noradrenaline or striatal dopamine content. At the time of biochemical assessment the behavioural studies showed enhanced sensitivity to apomorphine and 2-(N,N-dipropyl)amino-5,6-dihydroxytetralin (parallel shift in dose-response curves to the left).

In the apomorphine climbing model haloperidol (0.0063–0.1 mg/kg i.p.), sultopride (0.125–2.0 mg/kg i.p.) and metoclopramide (0.5–2.0 mg/kg i.p.), given peripherally as pretreatments, were equipotent to antagonise climbing in both normal and mesolimbic denervated mice. In contrast, sulpiride and thioridazine were more potent in the denervated model (2.5–20.0 mg/kg i.p. and 0.625–10.0 mg/kg i.p. sulpiride, 2.0–10.0 mg/kg i.p. and 0.25–2.0 mg/kg i.p. thioridazine in normal and denervated animals respectively). As with the peripheral administration of typical neuroleptics, direct intra-accumbens fluphenazine (0.25–1.0 μg, acute stereotaxic injection under ether anaesthesia, 1 h recovery before apomorphine administration to induce climbing) was equipotent to reduce climbing in normal and denervated mice, whilst sulpiride was more potent following mesolimbic denervation (0.25–2.0 μg and 0.0625–0.5 μg in normal and denervated mice respectively). In the second behavioural model circling was induced following unilateral striatal electrolesions (1.0 mm anterior to bregma, 2.0 mm lateral and 3.5 mm below the skull surface, 1.5 mA/15 s). Dopamine agonists and antagonists were tested in mice with electrolesions alone or combined unilateral striatal electrolesion and bilateral 6-OHDA accumbens lesions. Apomorphine was more potent to cause circling in the combined lesion model. The atypical neuroleptic agents sulpiride and thioridazine more actively inhibited circling in mice with combined striatal and mesolimbic denervation, as compared to their inhibitory action in mice with electrolesions alone (20.0–40.0 mg/kg i.p. compared with 5.0–10.0 mg/kg i.p. sulpiride, 5.0–10.0 mg/kg i.p. compared with 1.25–5.0 mg/kg i.p. thioridazine), than typical agents such as haloperidol (0.025–0.1 mg/kg i.p. in both models).

The present data emphasises the importance of the mesolimbic nucleus accumbens for the control of climbing behaviour, and its joint involvement with the striatum in the circling phenomenon. Secondly, the data provides a clear indication that atypical neuroleptics such as sulpiride and thioridazine are more effective to inhibit dopamine function in denervated mesolimbic mechanisms, as compared with a non-denervated situation, than typical neuroleptics such as haloperidol and fluphenazine.

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## Glutamatergic pathways from the medial prefrontal cortex to the anterior striatum, nucleus accumbens and substantia nigra

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The medial prefrontal cortex is thought to regulate subcortical dopamine function (Carter & Pycock, 1978; Pycock, Carter & Kerwin, 1980), possibly via its projections to the striatum (Webster, 1961) nucleus accumbens, lateral and basal amygdaloid nuclei and substantia nigra (Beckstead, 1979). The transmitter of the corticostriatal tract is believed to be glutamate (McGeer, McGeer, Scherer & Singh, 1977). and so the possibility of frontal cortical glutamatergic efferents to other subcortical dopamine containing regions has been investigated.

Male Wistar rats were used (150–175 g). Bilateral electrolytic lesions (2 mA/10 secs) of the medial prefrontal cortex were made at A, 10.3, L ± 0.8, V + 1.0 mm (Konig & Klippel, 1963). The location of the lesion was subsequently verified histologically. Sham-operated and lesioned animals were killed 10 days after surgery. Glutamate and GABA uptake were measured in a $P_2$ synaptosomal preparation of tissue from the nucleus accumbens, anterior and posterior striatum, amygdaloid complex, and the substantia nigra (pars compacta and reticulata). Tissue

was pooled from 3 to 4 animals, and uptake measured at 37°C using [$^{14}$C]-glutamate (285 mCi/mMole) or [$^{3}$H]-GABA (60 Ci/mMole; Radiochemical Centre, Amersham) diluted with cold amino acid to give a final concentration of $5 \times 10^{-7}$ M. Blank values were obtained at 0°C. The amount of glutamate or GABA binding to the tissue, as opposed to uptake, was measured under identical conditions after freezing and lysis of the synaptosomal fraction. The binding of [$^{14}$C]-glutamate to control tissue represented a relatively large proportion of the apparent uptake (4–12%) and so a more accurate estimate of glutamate uptake was obtained by subtracting the amount of radioactivity bound to the tissue, from that retained in the synaptosomal preparation. The relative binding of [$^{3}$H]-GABA to the tissue under these conditions was negligible (< 1.4%).

In the lesioned animals [$^{14}$C]-glutamate uptake was reduced in the nucleus accumbens, anterior striatum and substantia nigra to 79, 87, and 79% of control levels respectively ($P < 0.01$). In these areas, there was respectively a 4, 1.5 and 10-fold increase in the binding of [$^{14}$C]-glutamate to the tissue ($P < 0.01$). Reduced uptake, or enhanced binding of [$^{14}$C]-glutamate were not noted in other areas. After correcting for binding [$^{14}$C]-glutamate uptake was reduced in the lesioned group to 66, 78 and 41% of control levels in the nucleus accumbens, anterior striatum and substantia nigra ($P < 0.001$). After such correction, there was still no reduction in [$^{14}$C]-glutamate uptake in the posterior striatum or amygdaloid complex of the lesioned group.

[$^{3}$H]-GABA uptake was reduced in the nucleus accumbens of the lesioned group (to 84%, $P < 0.01$) but not in any other area. There was a two-fold increase in the binding of [$^{3}$H]-GABA to tissue from this nucleus ($P < 0.01$). This did not alter the extent of the reduction in [$^{3}$H]-GABA uptake.

While the possibility of aspartate-containing neurones cannot be precluded, the results support the contention that glutamate is the neurotransmitter of the corticostriatal tract, and also suggest the presence of glutamatergic pathways from the medial prefrontal cortex to the nucleus accumbens and substantia nigra.

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## Kainate-induced circling behaviour in rats

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Kainic acid, a conformationally restricted analogue of glutamate, is a potent excitotoxic agent (Olney, Rhee & Ho, 1974) which, on direct injection into striatum of rats, causes destruction of intrinsic cell bodies whilst generally sparing afferent nerve endings and fibres of passage (Olney, Sharpe de Gubareff, 1975). This causes focal seizures and circling behaviour (Nicklas, Duvoison & Berl, 1979).

The mechanism of circling caused by unilateral intrastriatal kainate has been investigated in female Cobb Wistar rats (150–200 g).

Unilateral intrastriatal injection of kainic acid (0.5 μg to 5.0 μg in 1 μl normal saline, adjusted to pH 7 with 2 N sodium hydroxide) caused dose-dependent circling behaviour. Using a standard dose of kainic acid (2 μg) which caused a consistent circling response, three phases of behaviour were observed. Initial ipsiversive rotation lasting up to 2 h was followed by contraversive rotation lasting between 10 to 36 h which was succeeded by inconsistent ipsiversive circling. Injections of normal saline produced none of these behavioural effects.

Similar intrastriatal injections of L-glutamic acid

(91.3 μg and 182.6 μg in 1 μl normal saline, adjusted to pH 7 with 2 N sodium hydroxide) and the monosodium salt of L-glutamic acid (93.5 μg and 187 μg in 1 μl normal saline) induced no postural asymmetry or circling behaviour. Injection of monosodium glutamate (187 μg) in the same solution (total volume 1 μl) with kainic acid (2 μg) produced a lower rate of contraversive rotation than observed in animals receiving kainate alone ($P < 0.05$).

To test the role of synaptically released glutamate in kainate-induced circling, the effect of unilateral ablation of the corticostriate glutamate pathway was examined by removal of frontal and parietal cortical afferent inputs to the striatum (for method see McGeer, McGeer, Scherer & Singh, 1977). Following such cortical lesions contraversive circling to kainate was reduced by 75% at 6 h post injection ($P < 0.0125$; $n = 6$) and was abolished or reversed after 8 h ($P < 0.0025$; $n = 6$).

Ipsilateral electrocoagulation of the strio-nigral pathway (König & Klippel, 1963; A 3.8, L 2.1, V −2.2) prolonged the period of ipsiversive rotation and markedly attenuated the contraversive rotation rate 2 h to 10 h after kainate injection ($P < 0.05$; $n = 11$).

The involvement of the ascending nigro-striatal dopaminergic system in kainate induced circling was also examined. Haloperidol (1 mg/kg i.p., 30 min previously) abolished the initial ipsiversive rotation and markedly reduced the rate of contraversive rotation between 5 h and 8 h after kainate ($P < 0.05$; $n = 14$). Postural asymmetry was more marked, however, causing the rats to rotate in tighter circles. 6-Hydroxydopamine (8 μg/3 μl) lesions of the ascending medial forebrain bundle ipsilateral to the kainate injection, abolished initial ipsiversive rotation, increased contraversive rotation rate up to 4 h ($P < 0.05$; $n = 6$), but subsequently failed to modify the circling when compared to kainate alone.

The putative glutamate antagonists glutamic acid dimethyl ester (250 μg/μl normal saline), 2-amino-4-phosphonobutyric acid (50 μg/μl normal saline), diaminopimelic acid (50 μg/1 μl normal saline) and DL-α-methyl-glutamate (20 μg/1 μl normal saline) injected into the anterior caudate in the same solution as kainate, or in the case of glutamic acid diethylester (50 μg and 250 μg/1 μl normal saline) 30 min prior to kainate did not modify circling behaviour over the 10 h observation period.

In conclusion, circling behaviour produced by injections of kainic acid into the anterior caudate putamen of rats appears to require an intact corticostriatal glutamate pathway and may involve a cooperative action with pre-synaptically released glutamate. The ascending dopaminergic systems are not involved, but circling appears to be mediated, at least in part, through the strio-nigral pathways.

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## 2-Amino-5-phosphonovalerate (2APV), a highly potent and specific antagonist at spinal NMDA receptors


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Receptors for excitatory amino acids have recently been classified into different types (Evans, Francis, Hunt, Oakes & Watkins, 1979; Davies & Watkins, 1979; McLennan & Lodge, 1979). Receptors of one type (NMDA receptors) are activated by the potent excitant, N-methyl-D-aspartate (NMDA) and blocked by a range of monoamino and diamino dicarboxylic acids, including D-α-aminoadipate (DαAA). The same antagonists block synaptic excitation in the vertebrate spinal cord, and a parallelism has been demonstrated between the relative abilities of these substances to block synaptic excitation and their relative abilities to block NMDA-induced responses. It is considered likely that NMDA receptors are transmitter receptors which mediate the effects of an excitatory amino acid

transmitter. We report here the discovery of a new, highly specific NMDA receptor antagonist, (±)-2-amino-5-phosphonovalerate (2APV) which is considerably more potent than DαAA and related substances.

Experiments were conducted on neurones in the spinal cord of the frog (*Rana temporaria*) *in vitro*, and of the cat *in vivo*. In the frog experiments, the effects of antagonists were measured on the amplitude of the motoneuronal depolarizations induced by various amino acid excitants present in the superfusion medium, or evoked by dorsal root stimulation. Tetrodotoxin ($10^{-7}$ M) was present in the medium in the case of the amino acid-induced responses to minimize indirect effects of the agonists and antagonists. Conventional iontophoretic techniques were used for the experiments on cat spinal neurones, the effects of the antagonists being determined on the frequency of spike discharge of cells excited by exogenous amino acids or by synaptic activation.

2APV depressed responses to NMDA in both preparations with a potency approximately 15 times that of DαAA. It had little or no effect on responses to kainate or quisqualate which are preferential agonists for other types of excitatory amino acid receptors (Evans *et al.*, 1979; Davies & Watkins, 1979; McLennan & Lodge, 1979). Responses induced by L-aspartate and L-glutamate, which are less selective agonists, were antagonized to intermediate degrees. 2APV had no action on ACh-induced excitation of cat Renshaw cells, or the cholinergic excitation of these cells evoked by ventral root stimulation. However, dorsal root-evoked excitation of these cells, which is probably mediated by an excitatory amino acid transmitter, was very effectively blocked by 2APV. This substance may therefore prove to be a valuable tool in studies of amino acid-mediated synaptic excitation in the vertebrate central nervous system.

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## Amino acid sensitivity of isolated spinal root fibres from the rat

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Neutral amino acids have been reported to depolarize primary afferent fibres and motoneurones as recorded in spinal roots of isolated hemisected spinal cords from immature (3–9 day old) rats (Evans, 1978). In the present experiments it was found that isolated ventral root fibres from these preparations were depolarized by γ-aminobutyric acid (GABA, 0.01–1 mM) or glycine (0.05–1 mM). Thus depolarizing responses to GABA or glycine recorded previously in ventral roots (Evans, 1978) probably represented responses of fibres rather than motoneurones. This possibility is supported by the action of taurine (0.5 mM) which had only a weak depolarizing action, relative to glycine, on isolated ventral root fibres and produced hyperpolarization recorded in ventral roots attached to hemicords.

Glycine, in contrast to GABA, had only a weak depolarizing action on isolated dorsal root fibres. Isolated ventral roots were unaffected by the excitant amino acids kainate (1 mM) or N-methyl-D-aspartate (NMDA) (1 mM) but isolated dorsal roots were very sensitive to kainate (threshold 1 μM) whereas NMDA (1 mM) had little or no effect on them. Isolated dorsal roots from mature rats were sensitive to GABA (Brown & Marsh, 1978) but insensitive to kainate (1 mM) or NMDA (1 mM). Ventral roots from mature rats were insensitive to all these amino acids.

These observations support the suggestion that there are at least two distinct receptor types for excitant amino acids, characterized by the agonists kainate or NMDA (Evans, Francis, Hunt, Oakes & Watkins, 1979). Isolated spinal roots from the immature rat offer the possibility of discrimination between these receptor types.

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## Motor responses evoked by N-methyl-D-aspartic acid and kainic acid from substantia nigra and ventral tegmental area in the rat

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Since the ascending nigrostriatal and mesolimbic dopaminergic pathways are closely associated with the control of motor activity, potential neurotransmitter systems within the region of the midbrain dopamine cell bodies are of great interest. In this respect for example the inhibitory amino acids γ-aminobutyric acid and glycine have been well investigated (Arnt & Scheel-Krüger, 1979; James & Starr, 1979). However, although the possible neurotransmitter roles for L-glutamate and L-aspartate supraspinally have not been established (Johnson, 1978), there are few studies investigating the role of these excitatory amino acids in motor behaviour. We have used kainic acid and N-methyl-D-aspartic acid (NMDA) as tools for their proposed agonist actions at glutamate and aspartate receptors respectively (Watkins, 1978).

Bilateral guide cannulae (O.D. = 0.65 mm) were stereotaxically implanted above the ventral tegmental area (VTA) and substantia nigra (SN) in female Porton rats. Cannulae were placed above the pars compacta ($SN_c$; A 2.4, L ± 2.0, V − 2.0), pars reticulata ($SN_R$; A 1.6, L ± 2.0, V − 2.4) and VTA (A 2.2, L ± 0.5, V − 3.0; coordinates König & Klippel, 1963). Intracerebral injections of vehicle, kainic acid or NMDA (0.05–1 μg/μl 0.05 M phosphate buffer, pH 7.4) were made four days after surgery, the animals being manually restrained. Motor activity was recorded in an open field, while stereotypy and catalepsy were assessed and converted to a 0–4 rating scale.

Bilateral injection of either kainic acid (0.5 and 1 μg) of NMDA (0.05 and 0.1 μg) into the VTA significantly enhanced locomotor activity ($P < 0.05$, $P < 0.01$). Higher doses of these excitatory amino acids induced running seizures. Prior treatment of rats with fluphenazine (1 mg/kg i.p.) significantly reduced the enhanced motor response to the 0.5 μg dose of both kainic acid and NMDA.

Bilateral injection of 0.5 μg kainic acid and NMDA into the $SN_c$ evoked some increased motor activity ($P < 0.05$) together with periods of intense sniffing behaviour (stereotyped score 2; $P < 0.01$, Mann-Whitney U test). Pretreatment with fluphenazine blocked the locomotor component induced by both amino acids and reduced the intensity of sniffing (score 1). Unilateral injection of NMDA or kainic acid (0.5 μg) into the $SN_c$ produced a mild/moderate contralateral posture with occasional bursts of contraversive turning.

In contrast, application of NMDA (0.5 μg) bilaterally to the $SN_R$ induced sedation: 1 μg doses of both amino acids to this site caused animals to maintain cataleptic postures for periods up to 5 min ($P < 0.01$). Unilateral application of the excitatory amino acids to this site initially caused a strong contralateral posture although in conjunction with backward locomotion rats turned towards the side of injection.

The results from this study suggest NMDA- and kainate-sensitive sites within the rat SN and VTA. The differing motor effects observed when these two agents were applied to either the $SN_c$ or $SN_R$ may predict a dual motor response for excitatory amino acids within the SN as has been proposed for GABA (Reavill, Jenner, Leigh & Marsden, 1979).

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## Effect of dopamine on the release of [$^3$H]-glycine and [$^3$H]-taurine from the rat isolated retina

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There is evidence to suggest that dopamine and glycine may function as neurotransmitters in the rat retina (Marshall & Voaden, 1974; see Starr, 1978). In addition it has been suggested that taurine may also have a neurotransmitter role in this tissue (Pasantes-Morales, Klethi, Ledig & Mandel, 1972; Neal, Peacock & White, 1973). However, the possible interactions between these putative neurotransmitter candidates do not seem to have received attention. We have investigated the release of preloaded [$^3$H]-glycine and [$^3$H]-taurine from superfused rat retina, and have studied the effect of adding dopamine to the superfusing medium. The method used to study the release of preloaded [$^3$H]-transmitter from superfused rat retina *in vitro* was based on that described elsewhere (Kerwin & Pycock, 1979). Half retinae were superfused with Krebs-bicarbonate buffer, pH 7.4. In experiments using dopamine, ascorbic acid (0.01%) and pargyline (50 μM) were present to inhibit its metabolism.

A depolarising stimulus (60 mM KCl) increased the rate of spontaneous efflux of both [$^3$H]-glycine ($P < 0.02$, paired $t$ test) and [$^3$H]-taurine ($P < 0.01$) from the superfused rat retina. However, the release profiles of the two amino acids differed, the peak [$^3$H]-taurine release being consistently 6–8 min later than the peak [$^3$H]-glycine release. Omitting calcium from the superfusion medium, and adding EDTA (1 mM), abolished the potassium-stimulated release of [$^3$H]-glycine: the potassium-evoked release of [$^3$H]-taurine was reduced, although not abolished, in calcium-free medium.

Addition of dopamine (5 mM) to the superfusate inhibited the spontaneous release of both amino acids ($P < 0.05$): the effect on [$^3$H]-glycine release was antagonised by (+)-butaclamol (10 μM) ($P < 0.05$). Lower concentrations of dopamine (1 mM) had no significant effect. Further dopamine (5 mM) applied simultaneously with 30 mM KCl did not affect the delayed release of [$^3$H]-taurine, but dopamine did block this release if applied immediately after the depolarising stimulus.

In additional studies rat retina was incubated for 5 min with either [$^3$H]-glycine or [$^3$H]-taurine (0.2–2 μM) and radioactivity was accumulated with apparent $K_m$ values of 2.97 and 1.67 μM respectively indicating high affinity uptake mechanisms for both amino acids.

Distribution studies have localised glycine within a subpopulation of amacrine cells in the rat retina (Yates & Keen, 1976; Starr, 1978). It is possible that calcium-dependent release of [$^3$H]-glycine is from these cells: an action which may be regulated through dopamine mechanisms. However, the evidence for a neurotransmitter role for taurine is equivocal. The present results are difficult to interpret, but may suggest the existence of more than one pool of taurine in the rat retina.

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## Biosynthesis of substance P from [$^{35}$S]-methionine in isolated sensory ganglia

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There is increasing evidence to suggest that substance P (SP) may be the neurotransmitter utilised by certain primary afferent neurones (Otsuka & Konishi, 1976). One population of dorsal root ganglion (DRG) neurons contains immunoreactive SP in both its central and peripheral terminals, and also in its cell bodies, where the peptide is presumably synthesised (Hökfelt, Elde, Johansson, Luf, Nilsson & Arimura, 1976; Hökfelt, Kellerth, Nilsson & Pernow, 1975). The direct demonstration of SP biosynthesis in DRG would greatly strengthen the evidence for a neurotransmitter role for SP in these neurones. We now present evidence that isolated DRG incorporate [$^{35}$S]-methionine into SP characterised by immunoprecipitation followed by a two-step high performance liquid chromatography (HPLC) procedure.

Rat lumbar DRG were incubated at 37°C under oxygen in 0.5 ml of a HEPES-buffered medium containing Li[$^{35}$S]-methionine (100 μCi), for a period of 9 hours. The ganglia were then homogenised in acetic acid (2 M), centrifuged, and the supernatant lyophilised. Material soluble in barbital buffer, pH 8.6, was subjected to indirect immunoprecipitation with rabbit anti-SP serum followed by goat anti-rabbit IgG serum. Immunoprecipitates were redissolved in 0.2 M phosphate buffer pH 2.1, containing carrier SP, and the radiolabelled peptides separated by reverse-phase HPLC on Partisil 10 ODS. A peak of radioactivity, coincident with authentic carrier SP, was observed. Brief treatment of SP with hydrogen peroxide results in its conversion to SP sulphoxide, which has a shorter retention time on HPLC than the parent peptide. When fractions containing biosynthetically [$^{35}$S]-labelled SP were pooled after HPLC, treated with hydrogen peroxide and rechromatographed, a parallel conversion of carrier SP and of radiolabel to SP sulphoxide was observed.

A delay of 1–2 h was seen between addition of [$^{35}$S]-methionine and its appearance as [$^{35}$S]-SP. When DRG were incubated with [$^{35}$S]-methionine for 1, 2, 3 and 8 h, the radioactivity incorporated into SP was respectively 0, 439, 1084 and 1896 c.p.m. Synthesis of SP was blocked by cycloheximide (100 μM) and was reduced to 12% of control values in animals which had been treated neonatally with capsaicin, a drug which destroys a population of small-diameter chemosensitive primary afferent neurones which are thought to utilise SP as their transmitter (Jessell, Iversen & Cuello, 1978; Jancsó, Kiraly & Jancsó-Gábor, 1977).

These results provide evidence that SP synthesis in DRG occurs by a conventional ribosomal mechanism and that capsaicin may be a useful tool for the selective destruction of neurones responsible for this biosynthesis.

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## Atypical β-adrenoceptors on frog and chick erythrocytes

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Avian and amphibian erythrocytes have been used by a number of workers as model systems for the study of $\beta_1$ and $\beta_2$ adrenoceptors respectively (Vauquelin, Bottari, Kanarek & Strosberg, 1979; Pike, Limbird & Lefkowitz, 1979). The present study utilised direct receptor labelling techniques to compare the affinities of some highly selective adrenoceptor antagonists in these non-mammalian systems to those determined for $\beta_1$ and $\beta_2$ adrenoceptors of rat tissue, and suggests that these non-mammalian receptors may be different from those present in rat tissue.

Blood was obtained from 2–4 day old Ranger chicks, Esculenta frogs, and male Wistar rats (150–200 g). Erythrocyte membranes were prepared essentially as described by Charness, Bylund, Beckman, Hollenberg & Snyder (1976). Rat lung and cerebral cortex membranes were prepared as previously described (Rugg, Barnett & Nahorski, 1978; Nahorski, 1978). The binding of [$^3$H]-dihydroalprenolol ([$^3$H]-DHA) was performed as described by Nahorski (1978); non-specific binding (the binding remaining in the presence of 200 μM (−)-isoprenaline) was <25% of total binding in rat brain membranes and <10% in all other preparations.

[$^3$H]-DHA bound to membranes from all tissues in a manner indicative of an interaction with β-adrenoceptors. Thus, binding was of high affinity, saturable, reversible, and displaceable by drugs that interact with β-adrenoceptors. Non-selective β-adrenoceptor antagonists displaced [$^3$H]-DHA binding according to the law of mass action with very similar affinities in all tissues. On the other hand, displacement of [$^3$H]-DHA from rat lung and cerebral cortex membranes by selective $\beta_1$ or $\beta_2$ adrenoceptor agents deviated from law of mass action behaviour and could be resolved upon iterative curve fitting into two components corresponding to $\beta_1$ and $\beta_2$ sites. The affinities of selective antagonists for rat erythrocyte receptors were very close to those determined for the $\beta_2$ adrenoceptor component in rat lung and cortex (Table 1). However, the affinities of these drugs for the frog and chick erythrocyte β-receptors did not correspond to their respective affinities at $\beta_2$ and $\beta_1$ adrenoceptors of rat lung and cerebral cortex. These anomalies could not be explained by a heterogenous $\beta_1$ and $\beta_2$ adrenoceptor population present in erythrocyte preparations, since all drugs generated law of mass action displacement curves, indicating homogeneity of receptors.

Although the order of potencies of catecholamine agonists for frog and chick erythrocyte β-receptors suggests $\beta_2$ and $\beta_1$ adrenoceptor classification, respectively, the differences in affinities of antagonists suggest that these non-mammalian β-adrenoceptors may have phylogenetic differences in accessory binding sites to those present in the β-receptors of rat tissue and cannot therefore be classified as $\beta_1$ or $\beta_2$ adrenoceptors.

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**Table 1** Inhibition constants of agents interacting with $\beta_1$ and $\beta_2$ adrenoceptors from tissues of mammalian and non-mammalian origin

| Drug | *Rat Erythrocyte* ($\beta_2$) | *Rat Lung* ($\beta_1$) | *Rat Lung* ($\beta_2$) | *Rat Cerebral Cortex* ($\beta_1$) | *Rat Cerebral Cortex* ($\beta_2$) | *Chick Erythrocyte* | *Frog Erythrocyte* |
|---|---|---|---|---|---|---|---|
| | | | ($K_i$ nM) | | | | |
| (±) Atenolol ($\beta_1$) | 5200 | 40 | 5800 | 67 | 4500 | 930 | 2760 |
| (±) Practolol $\beta_1$) | 27,400 | 350 | 48,000 | 310 | 28,000 | 2180 | 5220 |
| (±) SL 75212 ($\beta_1$) | 202 | 3.6 | 208 | NT | NT | NT | 180 |
| (±) Metoprolol ($\beta_1$) | 353 | 21 | 450 | NT | NT | 220 | 365 |
| (±) OPC 2009 ($\beta_2$) | 26 | 4100 | 41 | 2600 | 41 | 6200 | 370 |
| (±) ICI 118.551 ($\beta_2$) | 1.1 | 107 | 1.7 | 160 | 3.8 | 38 | 9.4 |

$K_i$ was determined from the equation $K_i = IC_{50}/1 + S/K_D$, where S is the concentration of [$^3$H]-DHA in the assay and $K_D$ is the dissociation constant for [$^3$H]-DHA. $IC_{50}$ values were obtained from iterative curve fitting. The data are the mean of 3–8 experiments conducted in duplicate, s.e. mean were <10%.

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## An attempt to characterise the β-adrenoceptor mediating renin release in the cat

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Renin release from the kidney can be stimulated by activation of the β-adrenoceptors of the renin containing cells (Johns & Singer, 1974).

It is uncertain whether these β-adrenoceptors are like those of the heart ($\beta_1$-adrenoceptors) or like those of the peripheral vasculature ($\beta_2$-adrenoceptors). In this study a comparison has been made of the ability of two selective antagonists, atenolol, a $\beta_1$-adrenoceptor antagonist, and ICI 118, 551 (Bilski *et al.* 1979) a potent $\beta_2$-adrenoceptor antagonist, to block three different β-agonist responses, (a) isoprenaline induced increases in heart rate, (b) isoprenaline induced falls in blood pressure and (c) renal nerve mediated renin release.

Male cats were anaesthetized with sodium pentobarbitone and unilaterally nephrectomized. Renal blood flow was recorded using an electromagnetic flowmeter. The renal nerves were cut and prepared for stimulation. The vagi were sectioned in the neck. Plasma renin activity was measured before and following 10 min of renal nerve stimulation (15 volts, 0.2 msec) at a rate which reduced renal blood flow by 30% (frequencies between 2–7 Hz). A bolus intravenous injection of isoprenaline was given 10 min after the end of stimulation at a dose sufficient to increase heart rate by 40–50 bts/min.

In 11 cats nerve stimulation increased plasma renin from $2.22 \pm 0.47$ to $5.77 \pm 1.04$ ng $ml^{-1}$ $h^{-1}$, while isoprenaline ($0.136 \pm 0.012$ $\mu g.kg^{-1}$) increased heart rate, from $173 \pm 14$ to $215 \pm 14$ bts/$min^{-1}$, and decreased blood pressure from $132 \pm 6$ to $85 \pm 4$ mmHg. Administration of atenolol in 5 animals, up to 3.0 mg/kg, caused a dose related inhibition of the isoprenaline induced heart rate responses, with 50% inhibition being obtained at a mean dose of $1.84 \pm 0.63$ mg/kg, and a reduction of the nerve mediated renin release, with 50% inhibition of the response being achieved at a dose of $0.38 \pm 0.09$ mg/kg. However, even at the maximum dose of atenolol, there was no measurable inhibition of the isoprenaline induced falls in blood pressure. In six other animals administration of ICI 118,551, up to 1.0 mg/kg, caused a dose related inhibition of the isoprenaline induced depression of blood pressure, with 50% inhibition of the response being achieved at a dose of $0.042 \pm 0.012$ mg/kg. At the maximum dose of ICI 118,551 used there was no inhibition of the nerve mediated renin release, although there was a 43% reduction in the heart rate response to isoprenaline.

These results clearly show that atenolol inhibits both nerve mediated renin release and isoprenaline induced increases in heart rate in a similar dose-dependent fashion but ICI 118,551 had no effect on either of these responses. These findings support the suggestion that the β-adrenoceptors mediating renin release are more like those of the heart ($\beta_1$-adrenoceptors) than those of the peripheral vasculature ($\beta_2$-adrenoceptors).

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## Evidence for a single type of α-adrenoceptor on human platelets

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Amongst various tissues there are thought to exist two sub-types of the α-adrenoceptor, labelled $\alpha_1$ and $\alpha_2$ (Berthelsen & Pettinger, 1977), which have been shown to be preferentially stimulated by different agonists (Wikberg, 1978).

On human platelets adrenaline and noradrenaline act via an α-adrenoceptor to induce platelet aggregation (Mills & Roberts, 1967) and antagonise the stimulation of adenylate cyclase by $PGE_1$ (Jakobs, Saur & Schultz, 1976). Agonists at both $\alpha_1$ and $\alpha_2$-adrenoceptors have recently been shown to potentiate the aggregation response to ADP in a similar manner to adrenaline and noradrenaline (Grant & Scrutton, 1979). This study set out to differentiate the α-adrenoceptor sub-types of intact human platelets by a ligand-binding technique using [$^3$H]-dihydroergocryptine which is reported to bind to both $\alpha_1$ and $\alpha_2$-adrenoceptors (Miach, Dausse & Meyer, 1978).

Platelet rich plasma produced by centrifugation of blood from healthy volunteers was centrifuged at 1700 g for 5 min at 10°C and the platelets resuspended intact in incubation buffer (0.1% EDTA/150 mM NaCl; pH 7.5). These platelets were incubated for 20 min at 37°C with [$^3$H]-dihydroergocryptine (final concentration 3 nM) and varying concentrations of the α-adrenoceptor ligand under study in the presence and absence of phentolamine (final concentration 5 μM). Incubations were terminated by centrifugation at 6500 g for 1 min and the radio-activity bound to the cells was determined as described previously (Boullin & Elliott, 1979).

Total binding of [$^3$H]-dihydroergocryptine to intact platelets was progressively inhibited by phentolamine over the range $10^{-8}$–$10^{-6}$ M then reached a plateau at approximately 70% total bound [$^3$H]-dihydroergocryptine. At concentrations exceeding $10^{-5}$ M further inhibition of [$^3$H]-dihydroergocryptine binding occurred. Yohimbine similarly inhibited total [$^3$H]-dihydroergocryptine binding in the range of $10^{-9}$–$10^{-7}$ M to a plateau level which again represented approximately 70% total bound. Prazosin had no effect on [$^3$H]-dihydroergocryptine binding up to $10^{-7}$ M and no plateau was observed in the subsequent inhibition curve. Specific binding (Boullin & Elliott, 1979) was inhibited by both yohimbine and prazosin in a simple monophasic manner with Hill coefficients of approximately 1.0.

The absence of any biphasic inhibition of specific binding, as observed in rat brain (Miach, Dausse & Meyer, 1978), argues against the existence of two distinct α-adrenoceptors in human platelets. Phentolamine binds to both α-adrenoceptor sub-types whereas prazosin and yohimbine are preferential antagonists at $\alpha_1$- and $\alpha_2$-adrenoceptors respectively (Doxey, Smith & Walker, 1977). Hence it appears that the α-adrenoceptor identified by [$^3$H]-dihydroergocryptine binding on intact human platelets resembles the $\alpha_2$- rather than the $\alpha_1$-type. The relative affinities [$K_i$ (compound)/$K_i$ (adrenaline)] of clonidine (0.057), oxymetazoline (0.0096), methoxamine (37.3) and phenylephrine (2.3) support this hypothesis (Wikberg, 1978). However such interpretations should be made with caution as the human platelet α-adrenoceptor differs from other central and peripheral α-adrenoceptors in that the endogenous catecholamines adrenaline and noradrenaline have considerably greater potency on platelet aggregation and adenylate cyclase activity than synthetic agonists identified on other α-adrenoceptors (Jakobs, 1978).

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## *In vivo* α-adrenoceptor selectivity of WB 4101: a widely used $\alpha_1$-adrenoceptor ligand

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WB 4101 is a potent selective $\alpha_1$-adrenoceptor antagonist *in vitro* (Mottram & Kapur, 1975; Dubocovich & Langer, unpublished observations), widely used in receptor binding studies to characterise $\alpha_1$-adrenoceptors in the periphery (Raisman, Briley & Langer, 1979) and CNS (U'Prichard, Greenberg, Snyder, 1979). However, relatively little is known of the selectivity of WB 4101 *in vivo*. Consequently we have now compared its effects in anaesthetized dogs to prazosin, a selective postsynaptic $\alpha_1$-adrenoceptor antagonist (Cambridge, Davey & Massingham, 1977), and yohimbine, a preferential presynaptic $\alpha_2$-adrenoceptor antagonist (Weitzell, Tanaka & Starke, 1979).

Antagonism of the diastolic pressor responses to noradrenaline (NA, 1 μg/kg) and adrenaline (Ad, 1 μg/kg) was used as an index of postsynaptic antagonism and reversal of clonidine's inhibition of the tachycardia produced by stimulation of the ansa subclavia (0.25–2.0 Hz, 1 ms, 10v for 1 min), as an index of presynaptic antagonism. Clonidine produced a frequency-related inhibition of the tachycardia elicited by nerve stimulation, (97.2% at 0.25 Hz and 29.3% at 2.0 Hz).

Prazosin in doses up to 100 μg/kg had no effect on clonidine's inhibition of the tachycardia produced by ansa stimulation, but blocked the NA and Ad induced pressor responses. The NA pressor response was less antagonised than that to Ad, ($42.2 \pm 4.2\%$ and $83.0 \pm 8.7\%$ respectively, $P < 0.05$) at 100 μg/kg of prazosin.

In contrast to prazosin, yohimbine (1–30 μg/kg), had no significant effect on the pressor responses to injected catecholamines. The inhibition by clonidine of the tachycardia produced by nerve stimulation was reversed at all frequencies by yohimbine (3 μg/kg). Furthermore, yohimbine (30 μg/kg) significantly potentiated the response to stimulation at 0.5 and 1 Hz above control levels.

WB 4101 (10 μg/kg) significantly antagonised the pressor responses to NA and Ad. The NA pressor response was less reduced than that to Ad ($26.8 \pm 7.4\%$ and $56.5 \pm 4.5\%$ respectively at WB 4101 300 μg/kg, $P < 0.05$). The inhibition by clonidine of the tachycardia induced by nerve stimulation was significantly reversed by WB 4101 (30 μg/kg). The responses to 0.5 and 1 Hz stimulation were potentiated above control levels by 300 μg/kg of the drug.

WB 4101 exhibited only approximately a three-fold separation between threshold doses for pre and postsynaptic effects and this contrast with prazosin where approximately a hundred-fold separation was observed. Furthermore, WB 4101 was approximately 10-fold less potent than yohimbine in blocking presynaptic receptors and 10–30 fold less potent than prazosin in blocking postsynaptic receptors in the dog.

In conclusion, the reported *in vitro* selectivity of WB 4101, unlike that of prazosin, cannot be demonstrated *in vivo* under these experimental conditions, and consequently great care must be taken when extrapolating *in vitro* selectivity data to *in vivo* situations.

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## An analysis of the effects of mianserin on adrenergic mechanisms

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In the cardiovascular system of the pithed rat, the antidepressant drug mianserin (1–3 mg/kg) has been postulated to interfere with adrenergic neurotransmission by a combination of blockade of the neuronal uptake of noradrenaline (NA) and antagonism at both pre- and post-junctional α-adrenoceptors (Robson, Antonaccio, Saelens & Liebman, 1978; Cavero, Gomeni, Lefèvre-Borg & Roach, 1979). It is not clear, however, which effect, if either, would be dominant at low doses.

We have now approached this problem using techniques which should differentiate between the above three mechanisms since a drug possessing such qualities will produce complex net effects which are the resultant of physiological and pharmacological antagonism (Docherty & McGrath, 1979a, b).

(1) Blockade of the neuronal uptake of NA was assessed on the cardiac chronotropic response in the pithed rat; as potentiation of (a) the response to a single stimulus applied to the cardioaccelerator fibres and of (b) the response to NA; and as inhibition of (c) the response to tyramine; α-adrenoceptors are not involved in these responses.

(2) Blockade of post-junctional α-adrenoceptors was assessed, on the diastolic arterial pressure of the pithed rat, as inhibition of responses to phenylephrine ($\alpha_1$-adrenoceptor agonist) or to xylazine ($\alpha_2$-adrenoceptor agonist) (see Docherty, MacDonald & McGrath, 1979).

(3) Blockade of pre-junctional α-adrenoceptors cannot be assessed reliably on adrenergic transmission if blockade of NA uptake is present. This was, therefore, determined as antagonism of the inhibitory effect of xylazine on the 'non-adrenergic' contraction of prostatic portions of bisected vas deferens to single supramaximal stimuli, *in vitro* (Docherty *et al*, 1979). Rats were reserpinised (2 mg/kg, i.v., 18 h) to eliminate interference from adrenergic mechanisms (Booth, Connell, Docherty & McGrath, 1978).

Results were as follows.

(1) Mianserin produced dose-dependent changes of cardioaccelerator responses: (a) single pulse stimulation: response increased; threshold, mianserin (0.01 mg/kg); equivalent effects by mianserin (0.1 mg/kg), desipramine (0.01 mg/kg) or cocaine (3.3 mg/kg); neither yohimbine (0.1–1 mg/kg) nor prazosin (1 mg/kg) produced this effect. (b) NA (0.2 μg/kg): response increased; threshold, mianserin (0.1 mg/kg); equivalent effects by mianserin (1 mg/kg) or desipramine (0.1 mg/kg). (c) tyramine (10 μg/kg): response decreased; threshold, mianserin (0.1 mg/kg); equivalent effects by mianserin (0.1 mg/kg) or cocaine (3.3 mg/kg).

(2) The pressor response to xylazine (10 μg/kg) was reduced by yohimbine (1 mg/kg) but not by mianserin (1 mg/kg) or prazosin (1 mg/kg). The pressor response to phenylephrine (10 μg/kg) was abolished by prazosin (1 mg/kg) but was reduced by only 50% by mianserin (1 mg/kg) or yohimbine (1 mg/kg).

(3) In the prostatic portion of reserpinised vasa, the inhibitory effect of xylazine on the nerve-induced contraction was significantly reduced by yohimbine (0.1 μM) but not by mianserin (0.1 μM).

It is concluded that at low doses (0.01–0.1 mg/kg) mianserin can block the neuronal uptake of NA. At higher doses (1–5 mg/kg) some antagonism at $\alpha_1$-adrenoceptors is found, which is equivalent to the effect of yohimbine. At $\alpha_2$-adrenoceptors, however, whether they are located pre- or post junctionally, mianserin has little antagonist effect.

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## Cardiovascular actions of the inhibitory material extracted from the bovine retractor penis

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The bovine retractor penis receives a dual innervation: in addition to motor noradrenergic fibres, there are inhibitory fibres whose transmitter is unknown (Klinge & Sjöstrand, 1974; Ambache, Killick & Zar, 1975). We have recently described a method (based on that originally described by Ambache *et al.* 1975) for preparing a partially-purified extract of the bovine retractor penis, from which the main pharmacologically active contaminants, noradrenaline and ATP, have been removed. This extract mimics the effects of inhibitory nerve stimulation in both the bovine retractor penis and the rat anococcygeus muscles (Gillespie & Martin, 1978; Bowman, Gillespie & Martin, 1979).

When injected intravenously into the pentobarbitone-anaesthetised rat, the extract was found to be without effect on either blood pressure, or heart rate, nor did it affect the rate or force of beating of isolated atria. While the experiments on the anaesthetised rat suggested that the extract lacked any general vasodilator property, it was possible, if it contained the transmitter released from the inhibitory nerves, that it would cause dilatation of the penile arterial system, since these specialised arteries relax in response to field stimulation (Klinge & Sjöstrand, 1974). The extract in fact produced relaxation of spiral strips of branches of the bovine penile artery, and also of rabbit aorta that had been made to contract with $BaCl_2$ or noradrenaline respectively. Evidence that the vasodilator substance and the substance that caused relaxation of the retractor penis were the same was derived from the following observations: assays on the rabbit aorta and the bovine retractor penis showed that in both preparations relaxant activity disappeared at 20°C with the same half-life, and reappeared in both after treatment with acid and subsequent neutralisation (see Gillespie & Martin, 1978). Heating the extract for 2 min in a boiling water bath irreversibly abolished its activity in both preparations.

The failure to lower the rat blood pressure in spite of a powerful relaxant effect on the rabbit aorta was not because the extract exerted a selective action on the smooth muscle of large conducting arteries which contribute little to peripheral resistance, since it was powerfully depressor in the Krebs-perfused mesenteric vessels of the rat and in the Krebs-perfused rat hindquarters, after vascular tone had been raised with adrenaline or noradrenaline. The finding that the material was depressor in the perfused rat hindquarters and in mesenteric vessels, and yet was inactive by intravenous injection in the intact rat, suggested either the lungs or the blood as the site of inactivation. However, the extract was also inactive when injected directly into the left atrium. This observation eliminated the lungs, and pointed to the blood, as the main site of rapid inactivation. Subsequent *in vitro* experiments have confirmed that this is so. If this compound has any biological function, it is obviously not as a hormone, because of its rapid inactivation in blood.

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## A comparison of the effect of (±)-propranolol, hexamethonium and sodium nitroprusside on the preganglionic sympathetic activity of the cat

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Central and peripheral mechanisms have been proposed to explain the antihypertensive action of β-adrenoceptor antagonists (Scriabine, 1979). In order to differentiate between these mechanisms a preliminary comparison has been made of the effect of (±)-propranolol with that of two peripherally acting drugs, hexamethonium and sodium nitroprusside on preganglionic sympathetic activity.

Cats were anaesthetized with a mixture of α-chloralose (70 mg/kg i.v.) and pentobarbitone (12 mg). Atropine (1 mg i.v.) was also given, the animals vagotomized and artificially ventilated after paralysis with gallamine. A pneumothorax was performed and expired $CO_2$ was kept near 4%. Simultaneous recordings were made of blood pressure, heart rate, femoral blood flow (from which conductance was derived) and sympathetic nerve activity. The method for recording sympathetic activity was that developed by Zaimis, Zanchetti, Malliani, Lombardi & Ramage (1978) which enables continuous recording over several hours from filaments of the third or fourth thoracic communicans. The nerve fibres used were those that responded to a carotid occlusion. Drugs were given by single injections into the jugular vein or by slow infusion into the brachial vein.

Injections of hexamethonium (0.5 mg/kg) in five animals caused a fall in BP (53 ± 26 mm Hg; mean ± s.e. mean) accompanied by a rapid rise in sympathetic activity (62 ± 22%; expressed as a percentage of background). Propranolol (0.5 mg/kg) injections in six animals caused a smaller fall in BP (21 ± 6 mm Hg) and a small but delayed rise in sympathetic activity (21 ± 8%). Both drugs increased femoral arterial conductance, this being considerably greater for hexamethonium.

Saline was infused in five animals (4 ml $kg^{-1}$ $h^{-1}$) over a period of 3 hours. Blood pressure remained almost unchanged (144 ± 6 mm Hg to 140 ± 8 mm Hg) but sympathetic activity gradually increased (41 ± 21%), accompanied by a slight increase in heart rate and a decrease in femoral conductance. Infusion of propranolol (4 mg $kg^{-1}$ $h^{-1}$) increasing stepwise to give a total dose of 17 mg/kg in five animals (starting at least 20 min after an initial injection of 0.5 mg/kg had been made) caused a gradual fall in BP from 121 ± 12 mm Hg to 93 ± 8 mm Hg over the same period. At the same time, sympathetic activity increased by 91 ± 37% and was accompanied by a slight decrease of the femoral conductance. Carotid occlusion at hourly intervals elicited no change in response. Finally, sodium nitroprusside was infused in two animals over a period of 3 h in a final dose of 200 μg/kg. Blood pressure fell in both experiments from 128 mm Hg to 116 mm Hg; heart rate increased from 203 to 248 beats/min and femoral conductance decreased. These effects were accompanied by a large increase in sympathetic activity (198%).

These results suggest that propranolol, administered in the form of a single i.v. injection, does modify the reflex compensation of the accompanying fall in blood pressure although the prolonged antihypertensive effect does not seem to be due to a central mechanism.

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## Postsynaptic location of $\alpha_2$ adrenoceptors in vascular smooth muscle

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Alpha adrenoceptors can be divided into two types on the basis of agonist and antagonist potencies; classical postsynaptic $\alpha_1$-receptors and $\alpha_2$-receptors which appear to be presynaptically located at least in some peripheral tissues (Starke, Montel and Endo, 1975). However, recent evidence, using radioligand binding techniques (U'Pritchard & Snyder, 1979) and classical pharmacology (Drew & Whiting, 1979) suggests a postsynaptic location for receptors, similar to $\alpha_2$ receptors.

We have studied the ability of a range of α-adrenoceptor antagonists to block the rise in mean arterial pressure (MAP) caused by injection of the following α adrenoceptor agonists: phenylephrine which is an $\alpha_1$ agonist, noradrenaline which acts on both $\alpha_1$ and $\alpha_2$ receptors, clonidine which is more active on $\alpha_2$ receptors, and guanabenz which has almost wholly $\alpha_2$ agonist properties (Berthelsen & Pettinger, 1977; Docherty, MacDonald & McGrath, 1979). The α blockers selected were the $\alpha_1$-antagonist prazosin, the mixed $\alpha_1/\alpha_2$-antagonist phentolamine and the predominantly $\alpha_2$-antagonist yohimbine.

All drugs were administered intravenously to male New Zealand white rabbits. Arterial pressure was measured directly from a catheter in the central artery of the ear. Blood pressure was measured before and 20–30 min after antagonist administration.

Phentolamine (1.0 mg/kg and 5.0 mg/kg) produced similar shifts in the dose response curves to phenylephrine (25–500 μg) and noradrenaline (2.5–50 μg). Prazosin (0.1 mg/kg and 0.5 mg/kg) blocked the pressor response to phenylephrine more effectively than that of noradrenaline while yohimbine (1 mg/kg) blocked that of noradrenaline preferentially.

Intravenous injection of clonidine (30 μg/kg) increased MAP by 27 ± 4 mmHg. Phentolamine was more effective than prazosin in blocking this pressor response when antagonist doses which gave a similar degree of postsynaptic $\alpha_1$ blockade were compared. Intravenous phentolamine (5.0 mg/kg) administered 20 min before caused a 78% reduction in the pressor response of clonidine to 6 ± 4 mmHg. After prazosin (0.5 mg/kg) the pressor response was reduced by 60% to 11 ± 4 mmHg. Intravenous injection of guanabenz (100 μg/kg) increased MAP by 26 ± 4 mmHg. Pretreatment with phentolamine (5.0 mg/kg) produced a 77% reduction in the pressor response to this agonist while prazosin (0.5 mg/kg) only reduced the response by 7% to 19 ± 4 mmHg.

Pretreatment of rabbits 48 h before with intravenous 6-hydroxydopamine (50 mg/kg) caused a reduction in the noradrenaline content of heart and spleen of 86% and 90% respectively compared to untreated controls. However, radioligand binding studies using tritiated clonidine (22.2 Ci/mmole) as the specific ligand showed no significant change in the affinity constant or maximum number of binding sites, ($B_{max}$). In spleen $B_{max}$ was 171 ± 42 fM/mg protein in controls and 146 ± 15 fM/mg protein in treated rabbits. Further there was no reduction in the pressor responses to the α adrenoceptor agonists in these animals; clonidine (30 μg/kg) caused a rise in MAP of 23 mmHg ± 4 and guanabenz (100 μg/kg) a rise of 24 mmHg ± 4.

Thus these studies provide further evidence for a postsynaptic location for $\alpha_2$-receptors but do not yet provide information in the relevance of these receptors or their role in the regulation of peripheral resistance and arterial blood pressure.

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## The effect of opiates on baroreceptor reflex sensitivity in the normotensive rabbit


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Narcotic analgesics decrease blood pressure and heart rate (HR) by a centrally mediated decrease in sympathetic tone and an increase in vagal activity, possibly as a result of facilitation of transmission of baroreceptor impulses (Laubie *et al*, 1974).

In this study the role of endogenous opiates in cardiovascular regulation and baroreceptor reflexes has been investigated using an opiate receptor antagonist naloxone and a stable encephalin analogue Tyr-D-Ala-Gly-Me-Phe-NH-$(CH_2)_2NMe_2$ (RX783016). RX783016 is a selective μ opiate receptor agonist, with low affinity for δ or K receptors (Bower *et al*, 1980).

Baroreceptor sensitivity was assessed in the anaesthetised rabbit (pentobarbitone 30 mg/kg) by measuring heart period (HP) in response to rises in mean arterial pressure (MAP) after bolus intravenous (iv)

injections of phenylephrine (5 and 10 μg/kg), falls induced by iv sodium nitroprusside (10 μg/kg) and controlled haemorrhage (10–40 ml). There was a highly significant linear relationship between HP and MAP for each experimental treatment ($r$ values ranged from 0.84 to 0.98). The slope of this linear regression was used as an index of baroreceptor reflex sensitivity.

Thirty min after the intracisternal administration of RX783016 (50 μg/kg), a dose which had no effect on MAP or HR, baroreceptor sensitivity was reduced to both doses of phenylephrine $2.0 \pm 0.3$ to $1.2 \pm 0.2$ ($P < 0.05$) and $2.9 \pm 0.8$ to $1.4 \pm 0.4$ ($P < 0.01$) ms/mm Hg respectively and to sodium nitroprusside (10 μg/kg) $1.6 \pm 0.3$ to $0.9 \pm 0.2$ ms/mm Hg ($P < 0.01$) and to blood withdrawal $1.2 \pm 0.4$ to $0.7 \pm 0.3$ ms/mm Hg ($P < 0.01$). These values represent the mean ± s.d. of responses in groups of 10 animals.

Fifteen min after an i.v. injection of naloxone (80 μg/kg) an increase in baroreflex sensitivity was observed after phenylephrine and sodium nitroprusside ($P < 0.01$). However this dose of naloxone by this route did not antagonise the reduction in sensitivity caused by intracisternal RX783016. A higher dose of naloxone (200 μg/kg i.v.) significantly attenuated ($P < 0.01$) the effect of RX783016. No change in baseline MAP or HR was observed after either dose of naloxone.

The present experiments demonstrate that endogenous and exogenous opiates may modify the baroreflex arc, by an action which may be mediated by μ receptors within the central nervous system. They do not permit a precise localisation of this effect and do not exclude participation of other opiate receptors in central blood pressure regulation.

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## The effect of exaprolol (MG 8823) a new β-adrenoceptor blocking agent, on epicardial ST-segment changes in a feline model of acute myocardial ischaemia

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There is a good deal of experimental evidence that β-adrenoceptor blocking drugs, administered prior to acute coronary artery occlusion, reduce both the extent (area) and degree (intensity) of myocardial ischaemic injury (e.g. Grayson, Irvine, Parratt & Cunningham, 1968; Reimer, Rasmussen & Jennings, 1973). Such histological evidence remains the 'gold standard' by which other less laborious techniques for the assessment of infarct size should be evaluated. One of the most often used of these is epicardial ST-segment mapping in dogs (Maroko, Kjekshus, Sobel, Watanabe, Covell, Ross & Braunwald, 1971) but the disadvantage of this model, at least in our hands, is the occurrence of, often fatal, ventricular arrhythmias following coronary artery ligation or occlusion (Marshall & Parratt, 1975). Further the 'short occlusion' technique in this species (Marshall & Parratt, 1977) relates only to pretreatment with drugs *before* the onset of acute myocardial ischaemia. We have examined the effects of ligation of the anterior descending branch of the left coronary artery on ST-segment changes measured simultaneously at 5 different epicardial sites in pentobarbitone-anaesthetized cats, using an adaptation of the method previously used in dogs (Marshall & Parratt, 1977). We have also determined the effects in this model of a potent new β-adrenoceptor blocking agent, MG 8823 (Carissimi, Gentili, Grumelli, Milla, Picciola & Ravenna, 1976) when administered 1 h after the onset of infarction.

As previously reported (Moore & Parratt, 1973) coronary artery ligation in cats seldom resulted in serious ventricular dysrhythmias; there were no significant changes in blood pressure, heart rate or LV $dP/dt_{max}$ although there was a significant increase in LVEDP (from $4.3 \pm 0.5$ to $6.9 \pm 1.0$ mm Hg; $P < 0.01$). Within 5–10 beats there was significant ST-segment elevation in epicardial electrocardiograms; this developed rapidly up to 5 min and reached a maximum at 40–60 minutes. Thereafter it was stable up to 2 h and declined gradually thereafter. After 1 h of ligation MG 8823 (1.0 mg/kg) was administered intravenously. This decreased heart rate (from $212 \pm 14$ to $168 \pm 7$ bts/min after 5 min) and LV $dP/dt_{max}$ (from $3220 \pm 430$ to $1880 \pm 260$ mm Hg) and

further increased LVEDP (from $5.8 \pm 0.6$ to $7.8 \pm 1.0$ mmHg); blood pressure was unchanged. Epicardial ST-segment elevation was reduced in 32 of 35 sites; the mean ST-segment elevation was $2.42 \pm 0.24$, $1.86 \pm 0.22$ and $1.70 \pm 0.16$ mV at 2, 3, and 4 h respectively in cats administered saline 1 h after ligation and $1.48 \pm 0.16$, $1.25 \pm 0.12$ and $0.93 \pm 0.1$ mV respectively in the drug-treated group ($P < 0.0001$).

We suggest that this experimental model is convenient and relevant for the laboratory assessment of the effect of drugs on infarct size.

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## Diazepam potentiates the coronary vasodilator actions of adenosine in anaesthetized dogs

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Although the coronary vasodilator actions of diazepam in experimental animals (Abel, Reis & Staroscik, 1970; Daniell, 1975) and man (Ikram, Rubin & Jewkes, 1973) are well documented, the mechanism involved is still incompletely understood. We recently reported that diazepam is capable of potentiating the effects of adenosine (1) on sympathetic transmission in rat vasa deferentia and (2) on guinea-pig cardiac muscle (Clanachan & Marshall, 1979). Since adenosine is a potent coronary vasodilator (Wolf & Berne, 1956), we have investigated the possible interaction between diazepam and adenosine on the coronary circulation of the chloralose-anaesthetized dog.

After induction with sodium thiopentone (25 mg/kg) five dogs were anaesthetized with intravenous chloralose (85 mg/kg) and positively ventilated with 100% $O_2$. Phasic coronary blood flow (CBF) was measured with a close-fitting Statham electromagnetic flow probe (2.0–2.5 mm) placed around the left circumflex coronary artery.

Intravenous injections of adenosine (1–100 μg/kg) produced consistent dose-dependent increases in CBF (Figure 1) without causing significant changes in heart rate or blood pressure. Higher doses of adenosine further increased CBF but caused decreases in blood pressure (5–15 mmHg) and slight bradycardia (<15 beats/min). Diazepam (1.0–4.0 mg/kg) produced transient (1–3 min) dose-dependent falls in blood pressure (10–36 mmHg) and more sustained (2–15 min) increases in CBF of between 13 and 62 ml/min. Heartrate was unchanged. Doses of diazepam which increased CBF also caused a significant potentiation of the coronary vasodilator actions of adenosine (Fig. 1) and increased their duration. In contrast equivalent volumes of diazepam solvent (propylene glycol 40%; ethanol 10% v/v) did not affect adenosine responses although the solvent itself produced a transient (1–2 min) increase in CBF ($73 \pm 9$ to $96 \pm 12$ ml/min; $P < 0.05$).

In conclusion, in doses which increase coronary blood flow, diazepam is capable of potentiating the coronary dilator actions of adenosine. Since diazepam is widely used in patients with ischaemic heart disease this action of diazepam warrants further investigation since other coronary dilators, like dipyridamole,

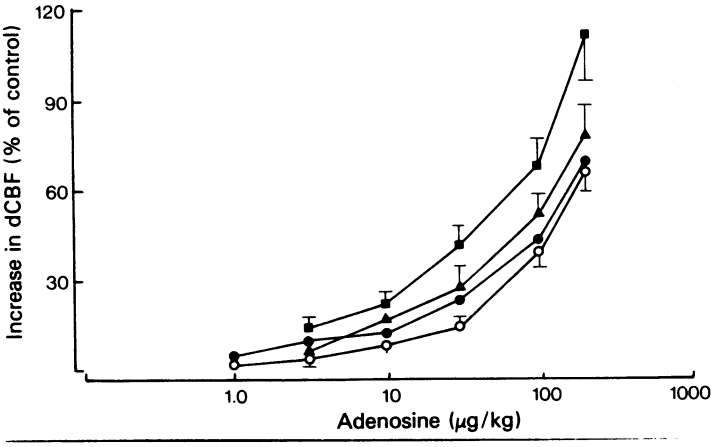


**Figure 1** Increases in diastolic left circumflex coronary blood flow (dCBF) produced by intravenous injections of adenosine alone (○) or after administration of diazepam 1.0 mg/kg (●), 2.0 mg/kg (▲) or 4.0 mg/kg (■). Each point is the mean ±s.e. mean of 5 determinations.

which act via an adenosine mechanism have been shown to further compromise blood flow in ischaemic myocardium by a 'coronary steal' mechanism (Marshall & Parratt, 1973).

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## Differentiation of the multiple effects of papaverine on the right and left auricles of guinea-pigs

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Left auricles were suspended in Chenoweth-Koelle solution, bubbled with 95% $O_2$ and 5% $CO_2$, at 37.5 ± 0·1°C and stimulated with 5ms pulses (of 1.5–3 V) at 1.6 Hz, for inotropic effects (IE). Right auricles beating spontaneously showed chronotropic effects (CE).

After two cumulative isoprenaline dose-response curves, a naive concentration of papaverine ($10^{-6}$–$10^{-4}$ M) was allowed to act for 1 h: washout of the drug was immediately followed by a third cumulative isoprenaline dose-response curve (to estimate potentiation or functional antagonism), at the acme of which papaverine was again applied (to accentuate negative changes). Antagonists, when used, were given prior to both papaverine and the third isoprenaline exhibitions.

Results are shown in Table 1.

Inotropic effects. At $10^{-6}$ M and $3.10^{-6}$ M papaverine, responses were positive and blocked by propranolol ($1–5.10^{-6}$ M). When the papaverine concentration was increased to $10^{-5}$ M, an additional positive inotropic effect (PIE) factor emerged which was propranolol- and promethazine-resistant ('Other', Table 1) and associated with potentiation of the

**Table 1** Papaverine actions on left (inotropic) and right (chronotropic) auricles of guinea-pigs

| *Papaverine concentration (M)* | *Positive inotropic or positive chronotropic factors* | | | *Negative inotropic effect (NIE)* | *Negative chronotropic effect (NCE)* |
|---|---|---|---|---|---|
| | *β-adrenoceptor mediated* | *Histamine-mediated** | *Other* | | |
| $10^{-6}$ | + | | | | − |
| $3.10^{-6}$ | + | | | | − |
| $10^{-5}$ | ++ | | + | | −− |
| $3.10^{-5}$ | ++ | + | + | − | −− |
| $10^{-4}$ | ++ | + | + | −− | −−− |

Positive inotropic or chronotropic effects: +, present; ++, marked.
Negative inotropic or chronotropic effects: −, present; −−, marked; −−−, very marked.
* Left auricle ($H_1$) blocked by promethazine ($3.10^{-6}$ M); right auricle ($H_2$) blocked by cimetidine ($3.10^{-6}$ M).

cumulative isoprenaline dose-response curve—probably phosphodiesterase inhibition. At $3.10^{-5}$ M and $10^{-4}$ M papaverine, an additional promethazine-sensitive PIE was added along with a membrane-stabilising negative inotropic effect (NIE). Isoprenaline cumulative dose-reponse curves, after $10^{-5}$ M and $3.10^{-5}$ M papaverine showed displacements to the left and increased maximum responses due to potentiation, whereas after $10^{-4}$ M papaverine the functional antagonism by the NIE minimized this latter action.

Chronotropic actions. The negative chronotropic effect (NCE) has a much lower threshold ($10^{-6}$ M papaverine) than the NIE (Table 1). So, the cumulative isoprenaline dose-response curves showed negligible displacements to the left and decreases in the maxima reached.

Most of the extant work on the cardiac effects of papaverine fails to recognise sufficiently its protean modes of action. The naive assumption that it is merely a phosphodiesterase inhibitor—perhaps with membrane stabilisation at higher concentrations—is fraught with difficulties and results in numerous tergiversations.

I am grateful to the Wellcome Trust for support.

## Differences between the functional antagonism of the positive inotropic and chronotropic responses to sympathomimetic amines by carbachol

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Antagonism of sympathetic increases in cardiac rate and force by the parasympathetic nervous system is an example of functional antagonism (Ariëns, Simonis & Van Rossum, 1964; Van den Brink, 1973) which occurs at a post-receptor cellular location possibly involving cAMP levels (Watanabe, McConnaughey, Strawbridge, Fleming, Jones & Besch, 1978). This present investigation was undertaken to further characterize this antagonism and to determine whether differences exist between the positive inotropic and chronotropic responses.

Guinea-pig isolated atria were set up at 38 or 25°C in Krebs-bicarbonate solution gassed with 5% $CO_2$ in oxygen (Broadley & Lumley, 1977). Paced left atria (2Hz, 5ms, threshold voltage + 50%) provided the inotropic responses and spontaneous right atria the chronotropic responses recorded on a Grass polygraph. Guinea-pigs were pretreated with reserpine (0.5 mg/kg i.p. at 24 h) and atria were incubated throughout with cocaine ($10^{-5}$ M), metanephrine ($10^{-5}$ M) and U-0521 ($10^{-4}$ M) to avoid neuronal and extraneuronal uptake and COMT degradation respectively. Cumulative concentration-response curves were constructed for isoprenaline, adrenaline or noradrenaline before and in the presence of carbachol. Increases in rate and tension were plotted as a percentage of the pre-carbachol maximum increase, correction being made for sensitivity changes from control experiments. Carbachol itself had negative inotropic and chronotropic effects at all concentrations employed. Shifts in concentration-response curves were expressed as the dose-ratio at the pre-carbachol $EC_{50}$.

At 38°C, carbachol ($3 \times 10^{-7}$ M) displaced the iso-

prenaline tension curve to the right (dose-ratio, 3.7) with a depression of the maximum to 82.6 ± 1.8%. The rate curve maximum was however depressed to 49.4 ± 5.4% ($n$ = 12). Adrenaline and noradrenaline were affected similarly. This greater sensitivity of the rate responses to antagonism by carbachol may be related to the rate selectivity of sympathomimetic amines at this temperature. This selectivity is reduced at 25°C (Duncan & Broadley, 1977), at which temperature the functional antagonism was examined next. The shift of the tension concentration-response curve to isoprenaline by carbachol ($3 \times 10^{-7}$ M) was virtually the same (dose-ratio, 6.5; max., 94.2 ± 5.0, $n$ = 8). However, with this concentration of carbachol at 25°C, spontaneous contractions continued in only 2/5 preparations in which the maximum rate was depressed to 20.3 ± 4.0%. At $10^{-7}$ M carbachol spontaneous activity persisted and the maximum rate increase was 47.7 ± 5.7% ($n$ = 15). Therefore the functional antagonism of rate responses was enhanced by cooling.

The effect of increasing the concentration of carbachol was next examined on the tension responses. At 38°C, there was no further shift of the concentration-response curve on increasing the carbachol concentration from $3 \times 10^{-7}$ M to $10^{-6}$ M (dose-ratio, 3.9; max., 87.3 ± 3.0, $n$ = 4) and $10^{-5}$ M (dose-ratio, 4.8; max., 79.6 ± 3.7, $n$ = 4). At 25°C, the shifts of the curves were similar for $10^{-7}$ M (dose-ratio, 5.8; max., 84.3 ± 6.6, $n$ = 4) and $3 \times 10^{-7}$ M (dose-ratio, 6.5; max., 94.2 ± 5.0, $n$ = 8) but further increases in carbachol concentration to $10^{-5}$ M (dose-ratio, 3.3; max., 97.3 ± 5.2, $n$ = 4) and $10^{-4}$ M (dose-ratio, 2.4; max., 103.9 ± 8.4, $n$ = 4) resulted in progressively less displacement. This lack of functional antagonism of the inotropic response at higher concentrations contrasts with the positive chronotropic response of isoprenaline and may reflect a fundamental difference in the post-receptor interaction between the sympathetic and parasympathetic systems for these two responses.

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## The effect of calcium on cyclic nucleotide levels in the rat isolated heart

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Changes in myocardial cAMP and cGMP are intimately linked to the regulation of intracellular calcium availability and cardiac contractility (Drummond & Severson, 1979). Conversely, biochemical studies have shown that calcium can affect the enzyme systems responsible for the synthesis and degradation of these nucleotides (Rasmussen & Goodman, 1977). The present study investigates the effects of extracellular calcium on cyclic nucleotide levels in an intact cardiac muscle preparation.

Hearts from male Wistar rats (200–280 g) were perfused at a constant coronary flow using a modified Langendorff technique. Contractility, at a resting tension of 2 g, was measured using a Devices UF1 transducer attached to the apex of the heart. After a 15 min stabilisation period the calcium content of the Kreb's-Henseleit solution was changed; 5 min later when the hearts had reached a new steady state they were freeze clamped at the temperature of liquid nitrogen. Samples of ventricle were then assayed for cAMP and cGMP.

Calcium over the range 0 to 2.43 mM produced an increase in contractility which was associated with a rise in ventricular cAMP and a variable effect on cGMP, this trend did not continue when the calcium level was raised to 4.92 mM (Table 1). Verapamil ($10^{-7}$ M) mimicked the effect of low extracellular cal-

**Table 1** The effect of extracellular calcium on cyclic nucleotides, contractility and heart rate in the isolated rat heart, means ± s.e. mean figures in parenthesis = number of hearts

| $Ca^{2+}$ conc mM | cAMP (pmoles/g wet wt) | cGMP (pmoles/g wet wt) | cAMP cGMP | Contractility (g) | Heart rate (beats/min) |
|---|---|---|---|---|---|
| 0.00 | 233 ± 14 (5) | 17.4 ± 1.3 (5) | 13.4 | 0.0 (5) | — |
| 0.30 | 277 ± 26 (5) | 16.0 ± 2.8 (5) | 17.2 | 1.1 ± 0.1 (5) | 246 ± 10 (5) |
| 0.61 | 337 ± 23 (5) | 37.1 ± 2.3 (5) | 9.9 | 4.6 ± 0.3 (15) | 234 ± 5 (15) |
| 1.23 | 422 ± 40 (7) | 19.6 ± 2.5 (10) | 21.5 | 9.1 ± 0.1 (20) | 239 ± 5 (20) |
| 2.46 | 620 ± 43 (10) | 15.8 ± 1.9 (15) | 39.2 | 10.7 ± 0.6 (20) | 232 ± 5 (20) |
| 4.92 | 461 ± 55 (5) | 51.1 ± 6.4 (5) | 9.0 | 10.8 ± 1.3 (5) | 254 ± 14 (5) |
| Verapamil ($1 \times 10^{-7}$ M) plus $Ca^2$ (1.23 mM) | 320 ± 22 (4) | 35.5 ± 13.0 (4) | 9.0 | 3.4 ± 0.1 (4) | 248 ± 6 (4) |

cium on the cyclic nucleotides, however, there was a difference in the profile of contractile activity when verapamil and low calcium were compared.

These results show that extracellular calcium is an important determinant of the cyclic nucleotide levels within rat cardiac muscle over the concentration range in which contractility is altered.

A. Daugherty is a University of Bath Research Fund student.

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## The effect of sodium thiosulphate upon some of the *in vitro* pharmacological properties of phenoxybenzamine

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Sodium thiosulphate pretreatment can prevent phenoxybenzamine (PB) from reducing the pressor response to adrenaline in rats (Nickerson, 1962). This interaction has been attributed to chemical neutralisation, by thiosulphate, of the highly reactive ethyleneiminium ion formed from PB in aqueous solution (Nickerson & Gump, 1949). We wished to determine whether the catecholamine-potentiating effects exerted by PB can also be attributed to ethyleneiminium ion.

Tissues were kept at 38°C in Krebs solution, gassed with 5% $CO_2$ in $O_2$ and containing ascorbic acid (0.1 mM). Sodium thiosulphate had no direct pharmacological effect on either tissue at any concentration used. In every experiment the PB/thiosulphate reaction was allowed to proceed for 30 min at 38°C before introduction to the tissue.

Isolated paired anococcygeus muscles from male Wistar rats (250 to 350 g) were mounted under 1 g resting tension. Sequential (−)-noradrenaline (NA) log concentration isometric response curves were constructed before and 1 h after incubation with PB. Table 1 shows the concentrations of reactants for effective neutralisation.

Four tracheal segments were isolated from each guinea-pig (300 to 600 g, either sex), opened and mounted under 150 mg resting tension. Cumulative log concentration isotonic response curves to NA or (−)-isoprenaline (ISO) were constructed before and 1 and 4 h after incubation of two of them with PB 1 μM. An aminophylline-induced maximum relaxation was superimposed on catecholamine curves. Table 1 shows that the potentiation of both NA and ISO by PB can be attributed to ethyleneiminium ion formation.

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**Table 1** Effect of thiosulphate on the modification by PB of responses to catecholamines

| Agonist | PB (nM) | Time (h) | Control Response | Paired Test Tissue Thiosulphate (μM) | Paired Test Tissue Response |
|---|---|---|---|---|---|
| | | | *Antagonism on the rat anococcygeus*[1] | | |
| NA | 0.75 | 1 | 23.7 ± 6.1 | 100 | 4.6 ± 3.4 |
| NA | 3 | 1 | 31.3 ± 6.7 | 100 | −1.0 ± 3.1 |
| NA | 12 | 1 | 69.8 ± 6.5 | 100 | 13.0 ± 1.9 |
| NA | 100 | 1 | 100 | 100 | 58.4 ± 6.2 |
| NA | 1000 | 1 | 100 | 1000 | 9.1 ± 3.4 |
| | | | *Potentiation on the guinea-pig trachealis*[2] | | |
| NA | 1000 | 1 | 0.88 ± 0.13 | 1000 | 0.08 ± 0.08 |
| NA | 1000 | 4 | 1.03 ± 0.34 | 1000 | −0.18 ± 0.12 |
| ISO | 1000 | 1 | 0.26 ± 0.06 | 1000 | −0.16 ± 0.1 |
| ISO | 1000 | 4 | 0.34 ± 0.07 | 1000 | −0.20 ± 0.07 |

[1] Response to PB on the anococcygeus was assessed as the depression (%) of the maximum response evoked by NA ($10^{-3}$ M).
[2] Response to PB on the trachealis was assessed as the difference in $\log_{10}$ concentration ratios between PB-treated and -untreated segments.
Each value is the mean ± s.e. mean of at least six observations.

## The rat isolated caecum

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Unlike the guinea-pig, the caeca of rodents lack the longitudinal muscle (*taenia coli*). The *taenia coli* of the guinea-pig has been studied extensively, however, the caecum of the rat has not been so studied. The following was undertaken in order to characterize the pharmacological receptors in the rat caecum.

The caecum was suspended in a 20 ml bath filled with Tyrode solution and gassed with 5% $CO_2$ in oxygen. The tissue developed spontaneous contractions which were reduced by SC.19220 (50 ng/ml–5 μg/ml), atropine (1 μg/ml–5 μg/ml) but not indomethacin (5 μg/ml) left in contact with the tissue for 30–60 minutes.

Prostaglandins ($PGE_1$, $PGE_2$, and $PGE_{2\alpha}$), acetylcholine, methacholine, carbachol, 5-hydroxytryptamine and neostigmine contracted the tissue and gave $pD_2$ values of: 9.65 ± 0.23; 9.10 ± 0.16; 7.2 ± 0.1; 5.9 ± 0.1; 7.6 ± 0.27; 6.9 ± 0.4; 7.9 ± 0.1 and 3.9 ± 0.3 respectively. Relative to $PGE_2$, the intrinsic activities (α) were $E_1$ = 1.12; $E_2$ = 1.0; $F_{2\alpha}$ = 0.92, and relative to acetylcholine, α were 1.0; 1.1 and 0.83 for acetylcholine, carbachol and methacholine respectively. Sc.19220 (0.5 μg/ml–5 μg/ml) reduced the effects of PG's, acetylcholine and methacholine to almost the same degree. On the other hand, indomethacin (0.25–5 μg/ml) reduced the effects of acetylcholine and neostigmine but enhanced the effect of $PGE_2$.

Atropine gave $pA_2$ values of 8.7 ± 0.22; 8.00 ± 0.50; and 7.90 ± 0.60 against acetylcholine, methacholine and carbachol respectively. The slopes of Schild's plots were 1.05, 0.87 and 0.95 respectively. These differences in $pA_2$ and slopes were not significant. The effects of PG's were not influenced by atropine. Atropine (10 ng/ml–100 ng/ml) reduced or completely abolished 5-HT-induced contractions. In the presence of atropine, 5-HT in concentrations higher than 2 μg/ml relaxed the tissue.

Noradrenaline, adrenaline, isoprenaline and salbutamol relaxed the caecum and gave $pD_2$values of: 7.2 ± 0.05; 7.0 ± 0.1, 8.0 ± 0.03 and 5.80 ± 0.06 respectively ($n$ in each case = 5). Intrinsic activities relative to noradrenaline were noradrenaline = 1.0, salbutamol = 0.96 and isoprenaline = 0.82. However, the concentrations producing maximal relaxations varied, thus noradrenaline ≅ $10^{-6}$, salbutamol ≅ $10^{-5}$ M and isoprenaline ≅ $10^{-7}$ M.

Phentolamine (10–100 ng/ml) shifted noradrenaline dose response curve but did not influence salbutamol

and isoprenaline curves. H35/25 significantly shifted salbutamol dose response curve more than that of isoprenaline and atenolol shifted the latter more than the former.

It is concluded that the rat caecum affords a simple method for screening drugs with actions on prostaglandin, muscarinic and beta-adrenoceptors.

We are grateful to Dr. John Pike and Dr. Bergstrom of Upjohn and Searle for the gifts of prostaglandins and SC.19220 respectively.

## Altered monoamine-mediated behaviours following bicuculline-induced convulsions

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When rats are given a single daily electroconvulsive shock (ECS) for 10 days they display enhanced behavioural responses following treatments which increase brain 5-hydroxytryptamine (5-HT) and dopamine (DA) function. Thus they show increased responses following administration of tranylcypromine (Tcp) plus L-tryptophan (L-Trp) or Tcp plus L-dopa or following 5-HT or DA agonists (see Grahame-Smith, Green & Costain, 1978).

We have now investigated whether similar alterations in these behaviours occur following bicuculline-induced seizures. (+)-bicuculline (0.375 mg/kg) was injected via a tail vein into male Sprague-Dawley rats (weight 150–200 g). A tonic-clonic seizure of 1–3 min duration was consistently produced.

Twenty-four h following the last of 10 daily convulsions the rats were given Tcp (5 mg/kg i.p.) followed 30 min later by either L-dopa (50 mg/kg i.p.) or L-Trp (75 mg/kg i.p.). The resultant activity was measured on Automex activity meters and compared to controls which had received saline i.v. in place of bicuculline. Enhanced behavioural responses were seen in the bicuculline treated rats following both Tcp/L-Trp and Tcp/L-dopa (Table 1).

When animals were tested 24h following a single bicuculline-induced convulsion, enhanced responses were seen only following Tcp/L-dopa (Table 1). Enhancement was also observed 48 h after the seizure, but not at 12h or 120 hours. A sub-convulsive dose of bicuculline (0.2 mg/kg) failed to produce enhanced responses. 24 h following a single seizure enhanced be-

**Table 1** Effects of bicuculline or electroconvulsive shock (ECS) induced seizures on monoamine-mediated behavioural responses

| *Treatment* | *Number of Experiments* *n* | *Total activity counts following amino acid administration* *Tcp/L-dopa* | *Tcp/L-Trp* |
|---|---|---|---|
| Saline × 10 | 3 | 2502 ± 558 | 3219 ± 710 |
| Bicuculline × 10 | 3 | 4582 ± 353† | 5467 ± 1084† |
| Saline × 1 | 7 | 3524 ± 427 | 3595 ± 406 |
| Bicuculline × 1 | 6 | 4794 ± 561† | 3896 ± 220 |
| Sub-convulsive Bicuculline × 1 | 3 | 2531 ± 258 | N.D. |
| Saline × 1 + Halothane | 4 | 3818 ± 361 | N.D. |
| Bicuculline × 1 + Halothane | 4 | 3270 ± 776 | N.D. |
| ECS + Halothane | 5 | 3168 ± 899 | N.D. |
| ECS on anaesthesia | 4 | 4910 ± 555* | N.D. |

Number of treatments given (× 1 or × 10). Tranylcypromine (Tcp; 5 mg/kg) given 30 min before L-dopa (50 mg/kg) or L-tryptophan (L-Trp, 75 mg/kg).
Activity counts (in groups of three rats) reported as mean ± s.d. for 60 min following L-dopa and 90 min following L-Trp, or 60 min following apomorphine (see data in text).
Different from saline injected controls: † $P < 0.01$; † $P < 0.001$.
* Different from halothane anaesthetised rats: $P < 0.01$.

havioural responses were also seen to apomorphine (2 mg/kg i.p.) {Saline: 1977 ± 494 (3); bicuculline: 2938 ± 202 (3)}.

When a single bicuculline-induced seizure was produced in halothane anaesthetised rats, no behavioural enhancement was seen 24 h later. Similarly no enhancement was seen after a single ECS (120v, 1s) given under halothane anaesthesia (Table 1). Enhancement was seen when a single ECS was given to unaesthetised rats (Table 1).

These data, taken with previous work with the inhalant convulsant flurothyl (Green, 1978) suggest that altered monoamine-mediated behavioural responses may be a consequence of tonic-clonic seizures produced by various different convulsant agents.

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## Effect of anaesthetic administration on the appearance of enhanced monoamine-mediated behaviours following electroconvulsive shock

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In a previous communication (Green & Nutt, 1980) it was shown that the behavioral responses which follow tranylcypromine (Tcp) and L-dopa were enhanced 24 h following a single bicuculline-induced convulsion. Enhancement was not seen when the convulsion was produced in halothane anaesthetized rats. We now report the effects of two anaesthetics on the enhancement of these behaviours which follows a convulsion produced by electroconvulsive shock (ECS).

A single ECS (125v, 1s) was given through ear-clip electrodes to male Sprague Dawley rats (weighing 150–200 g) following anaesthesia with halothane or methohexitone (5 mg/kg i.v.). Twenty-four h later the activity following administration of Tcp (5 mg/kg i.p.) followed 30 min later by L-dopa (25 mg/kg i.p.) was similar to that of rats given anaesthetic alone. When a single ECS was given to unanaesthetized rats the be-

**Table 1** Effect of anaesthetics on the enhancement of dopamine-mediated behaviour produced by electroconvulsive shock

| *Treatment* | *Number of Observations n* | *Activity during 60 min following L-dopa administration to Tcp treated rats* |
|---|---|---|
| Handled | 6 | 1903 ± 945 |
| ECS × 1 | 5 | 3482 ± 500† |
| Halothane × 1 | 3 | 1486 ± 371 |
| ECS × 1/Halothane | 2 | 1575 ± 321 |
| Methohexitone × 1 | 3 | 2501 ± 684 |
| ECS × 1/Methohexitone | 3 | 2228 ± 550 |
| Halothane × 5 | 6 | 1711 ± 722 |
| ECS × 5/Halothane | 6 | 3430 ± 667† |
| ECS × 1, Halothane 1 h later | 3 | 1880 ± 483 |
| ECS × 1, Methohexitone 1 h later | 3 | 2121 ± 935 |

Number of treatments given (× 1 or × 5). Methohexitone dose, 5 mg/kg i.v.
Results show activity counts recorded by Automex meters on groups of three rats and are reported as mean ± s.d.
† Different from appropriate control $P < 0.01$.

havioural responses 24 h later were markedly enhanced (Table 1). Enhanced responses were seen in rats given ECS during halothane anaesthesia providing that a single ECS was given daily for 5 days (behaviours being examined 24 h after the final electroshock (Table 1)).

Data suggested that seizure modification by the anaesthetics was not the explanation for the retardation of the enhanced responses since administration of halothane or methohexitone, 1 h after a convulsion induced without an anaesthetic prevented the appearance of enhanced DA-mediated responses 24 h later. Either single or repeated (5 days) anaesthesia administration did not alter DA-mediated responses, compared with untreated controls.

When ECS was given once daily for 10 days to halothane anaesthetized rats, enhanced monoamine-mediated behavioural responses occurred for up to 7 days following the last seizure (Green, Heal & Grahame-Smith, 1977). In contrast, enhanced responses were not present 3 days after a single ECS given to unanaesthetised rats. It seems that anaesthetics retard the development of the increased monoamine-mediated behavioural sensitivity and may do so by interfering in some way with the postictal changes involved in the production of the behavioural enhancement.

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## Bicuculline blocks 5-HT and GABA effects on rat hypothalamic neurones

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Bicuculline and its methochloride are usually and rightly described as selective antagonists of the effects of γ-aminobutyric acid (GABA) in iontophoretic experiments (Curtis & Johnston, 1974; Dray, 1975). However, in some situations bicuculline does not discriminate between the depressant responses produced by iontophoretic serotonin (5-HT) and GABA, e.g. on cat cerebral cortical neurones (Straughan, Alexander & Watson, 1971). This apparent lack of specificity has now been confirmed in recent iontophoretic studies with the potent water soluble GABA antagonist, bicuculline methochloride, on rat rostral hypothalamic neurones.

Adult female rats were anaesthetised with urethane and prepared for a dorsal approach to the hypothalamus as described previously (Mayer, 1979). Single unit action potentials were recorded from a glass microelectrode mounted to an adjacent 7-barrelled pipette containing combinations of DL-homocysteic acid (0.2 M), β-alanine (0.5 M), GABA (0.5 M), glycine (0.5 M), taurine (0.5 M), 5-HT bimaleate (0.2 M) (pH 3.5), dopamine (0.2 M) (pH 3.5) and bicuculline methochloride (10 mM) in 165 mM NaCl. The activity of quiescent and slow neurones was increased by constant iontophoretic application of homocysteate while inhibitory agonists were applied in a regular drug and time cycle by an automatic timer.

Bicuculline methochloride clearly antagonised the inhibitions evoked by 5-HT on 8/11 cells tested, at a time when GABA inhibitions were similarly reduced; but neither glycine nor dopamine-evoked inhibitions of the same neurones were affected. The time course of onset and recovery of the GABA and 5-HT antagonism was similar, and in both cases could be overcome by increasing the currents applied to the respective agonist barrels.

In agreement with the previous study on the cerebral cortex (Straughan *et al* 1971), 5-HT at concentrations up to $10^{-3}$ M had little effect on the efflux of [$^3$H]-GABA from slices of the rostral hypothalamus incubated *in vitro* suggesting that 5-HT does not act by releasing GABA *in vivo*. Further experiments are needed to elucidate the mechanism and significance of the antagonism of 5-HT by bicuculline methochloride in the hypothalamus and cerebral cortex.

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## Leptazol as a γ-aminobutyric acid antagonist

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There is evidence that the convulsant leptazol can antagonize neuronal depolarizations and the conductance increases evoked by γ-aminobutyric acid (GABA) (Nicoll & Padjen, 1976; Macdonald & Barker, 1977; Simmonds, 1978; Scholfield, 1979). This action of leptazol has now been investigated further.

Dose-related depolarizations to GABA receptor activation were obtained from slice preparations of rat cuneate nucleus (Simmonds, 1978, 1980). In this preparation, (±)-bicuculline is a competitive antagonist at the GABA receptor whereas picrotoxin antagonizes at a separate site within the GABA response mechanism (Simmonds, 1980). To investigate the site of action of leptazol, concentrations of leptazol (10 mM) (±)-bicuculline (6 μM) and picrotoxin (10 μM) have been tested alone and in combination against responses to the GABA analogue muscimol. All the antagonists caused parallel displacements of the muscimol dose-reponse curves to the right. The dose ratios obtained are given in Table 1.

The combination of leptazol + bicuculline gave a muscimol dose ratio which suggested that the two antagonists were acting at separate sites, i.e. a value similar to the product of the dose ratios obtained with each antagonist alone (6.05 × 8.27 = 50.0). The combination of leptazol + picrotoxin, however, gave a muscimol dose ratio which was not significantly different from that with picrotoxin alone. This suggests that leptazol and picrotoxin were interacting in some way. Comparison of these results with the Schild plot for picrotoxin against muscimol (Simmonds, 1980) indicates that leptazol (10 mM) was equipotent with picrotoxin (4.6 μM). If the two antagonists were acting at a common site, the addition of picrotoxin (10 μM) might be expected to have given a muscimol dose ratio similar to that for picrotoxin 14.6 μM, i.e. 10.7 (Simmonds, 1980). The lower value actually obtained is not incompatible with a common site of action and raises the possibility that leptazol may have attenuated the action of picrotoxin.

These results suggest that leptazol exerts its action as a GABA antagonist at the same site as picrotoxin in the depolarization response mechanism, rather than at the GABA receptor.

**Table 1** Muscimol dose ratios (mean ± s.e. mean) in the presence of antagonists

| *Leptazol* 10 mM | *Bicuculline* 6 μM | *Leptazol* + *bicuculline* |
|---|---|---|
| 6.05 ± 0.26 (8) | 8.27 ± 1.39 (6) | 46.8 ± 5.8 (14) |
| *Leptazol* 10 mM | *Picrotoxin* 10 μM | *Leptazol* + *picrotoxin* |
| 5.21 ± 0.32 (8) | 9.55 ± 1.44 (7) | 7.99 ± 0.55 (15) |

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## The effect of sodium valproate on single unit activity in the rat brain

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(Introduced by C.J. PYCOCK)

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Sodium valproate (VP) is a clinically effective anticonvulsant (Simon & Penry, 1975). The drug can inhibit several of the enzymes in the catabolic pathway for γ-aminobutyric acid (GABA: Whittle & Turner, 1978), thus elevating brain GABA concentrations. However, the time course of this effect is not compatible with its ability to rapidly prevent experimentally induced seizure (Schmutz, Olpe & Koella, 1979). The drug does depress cortical cell firing with a rapid onset of action that parallels its ability to prevent electroshock induced convulsion (Schmutz *et al.*, 1979). We have extended these observations to look at the effect of VP on spontaneous and GABA dependent mechanisms on single unit activity in the rat substantia nigra and neocortex. Conventional iontophoretic and recording techniques were used throughout.

Intraperitoneal injection of VP (100–400 mg/kg) produced a dose dependent depression of firing rate in the majority of cortical cells ($n = 28$) and cells of the substantia nigra ($n = 11$). The peak depression in firing rate occurred 4.7 min for cortical cells and 5.7 min for nigral cells after injection. The inhibitory effect of microiontophoretically applied GABA (0.5 M, pH 3.5, 10–60 nA) on 68 cortical cells was significantly enhanced ($P < 0.001$) and prolonged ($P < 0.001$) during the simultaneous ejection of VP (0.5 M, pH 8, 40–80 nA). At currents greater than 40 nA iontophoresed valproate alone often had a slight excitatory influence on cell firing rate. The inhibitory effect of iontophoretically applied muscimol (0.5 M, pH 3.5, 40–80 nA) on the firing rate of 17 cortical cells was also significantly potentiated ($P < 0.01$). However, inhibitory responses to iontophoretically applied glycine (0.5 M, pH 3.5, 50–80 nA) were unaffected by iontophoretically applied VP (11 cells). In order to pre-empt VP's ability to raise synaptic GABA levels (Whittle & Turner, 1978), one group of rats was pretreated with gabaculine (100 mg/kg) intraperitoneally 16 h before experimentation, to produce maximal elevation of brain GABA (Matsui & Kamioka, 1979). In these animals the ability of VP to potentiate GABA mediated inhibitory responses was still observed (22 cells). The ability of VP to potentiate responses to GABA and muscimol was apparent within 1 minute. Transsynaptic inhibitory responses were produced in cells of Deiter's nuclei ($n = 12$), substantia nigra ($n = 23$) and cerebral cortex ($n = 13$) by submaximal electrical stimulation of the cerebellar cortex, striatum and cerebral cortex respectively. Such inhibitory responses were identified as being GABA mediated since they were abolished by bicuculline methyl ester (26 mM in 165 mM NaCl, pH 3.5, < 20 nA). Intraperitoneal application (100–400 mg/kg) of VP produced no consistent effect on these inhibitory responses, although analysis of the responses were complicated by the depressant or excitatory effects *per se* of VP on cell firing.

These results point to an ability of VP to produce direct effects on postsynaptic inhibitory mechanisms which may be independent of its ability to inhibit enzymes in the catabolic pathway for GABA.

RWK is an MRC student.
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# GABA facilitates or inhibits the evoked release of [$^3$H]-noradrenaline from rat cerebellar cortex slices by an action at separate receptors

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We have recently reported the existence of a novel receptor for GABA on autonomic nerve terminals (Bowery & Hudson, 1979; Bowery, Doble, Hill, Hudson, Shaw & Turnbull, 1979) and in brain slices of the rat (Bowery, Hill, Hudson, Doble, Middlemiss, Shaw & Turnbull, 1980). Activation of this receptor decreases the evoked release of radiolabelled transmitter. Unlike the classical GABA receptor the novel site is insensitive to the antagonist bicuculline and is not stimulated by the GABA analogue 3-aminopropane sulphonic acid (3-APS). However, it is activated by the $\beta$-chlorophenyl derivative of GABA, baclofen, which is inactive at bicuculline sensitive sites (Curtis, Game, Johnston & McCulloch, 1974; Davies & Watkins, 1974). It has been reported (Arbilla & Langer, 1979) that GABA enhances the $K^+$-evoked release of [$^3$H]-noradrenaline from rat occipital cortex slices. This contrasts with the reduction we observed in rat cerebellar cortex slices. We have therefore made further studies and now report that GABA can also enhance the evoked release in cerebellar slices. The observed effect depends on the $K^+$ concentration used to stimulate output.

Batches of four sagittal slices of rat cerebellar cortex (0.25 mm thick) were incubated for 20 min at 32°C in Krebs'-Henseleit solution containing [$^3$H]-noradrenaline (0.8 μM 5 Ci/mmole Radioamersham) and then for a further 20 min in radioactive-free solution. Each batch was superfused at 0.5 ml/min on a sintered glass support and the tritium content of consecutive 4 min superfusate samples determined by liquid scintillation spectrometry. All solutions contained ascorbic acid (0.1 mM) and iproniazid (0.5 mM). Superfusion with high potassium (KCl added) was limited in each batch to three periods of 2 min (at 16 min intervals). When GABA or its analogues were present they were added 1 min before and during the second period of superfusion with high potassium.

The first addition of $K^+$ at 35 mM, 25 mM or 15 mM (final concentration) increased the overflow of tritium by 272.0 ± 28.2% ($n$ = 5), 146.7 ± 13.2% ($n$ = 20) and 44.5 ± 3.31% ($n$ = 17) over basal respectively. In experiments using 25 mM $K^+$ GABA (100 μM) and baclofen (100 μM) reduced the evoked release of tritium by 19.0 ± 3.70% and 18.1 ± 3.37% respectively (mean ± s.e. $n$ = 6 in each case $P < 0.01$ paired comparison with control slices from same animals). 3-APS (100 μM) did not reduce the output. On increasing the $K^+$ concentration to 35 mM similar results were obtained. Bicuculline methobromide (BMB 100 μM) failed to prevent the reduction by GABA or baclofen at either $K^+$ concentration.

When the added $K^+$ concentration was reduced to 15 mM, GABA (100 μM) enhanced the evoked release of tritium by 39.4 ± 5.04% ($n$ = 10 $P < 0.001$). A depression in evoked release was never observed. 3-APS (100 μM) now also increased the release to an even greater extent (60.3 ± 8.9% $n$ = 4) than GABA but by contrast, baclofen still depressed the evoked release. BMB (100 μM) abolished the enhancement produced by 3-APS but converted the increase produced by GABA into a reduction comparable with that produced by baclofen. It would seem therefore that GABA can enhance or reduce the $K^+$-evoked output of [$^3$H]-noradrenaline. The former effect is masked at higher $K^+$ concentrations and in the presence of BMB suggesting that separate receptors are responsible. 3-APS and baclofen are selective ligands for these respective sites.

A.D. is an MRC student.
D.R.H. is an SRC student.

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## Central inhibition by GABA of the release of vasopressin by carbachol in the rat

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Kühn (1974) found that the injection of carbachol into the third cerebral ventricle of the rat caused a decrease in urine flow and an increase in urinary osmolality, conductivity and electrolyte concentration. These effects were attributed to the release of vasopressin. The present experiments confirm by direct estimation of the hormone in urine that carbachol does release vasopressin and show that GABA, which was investigated as a possible inhibitory transmitter in the hypothalamo-neurohypophysial system, blocks the release.

Vasopressin was extracted by a modification of the method of Moran, Miltenberger, Shuayb & Zimmerman (1964) from 5 ml samples of urine collected from water-loaded rats under ethanol anaesthesia. The extracts were assayed for antidiuretic activity. In a typical experiment, the control rate of urine flow was 161 μl/min and the amount of vasopressin excreted in 5 ml of urine was 42 μU. After the injection of 40 ng (2 μl) carbachol into a lateral ventricle (i.vent), the mean rate of flow was reduced to 63 μl/min over a period of 30 min and the amount of vasopressin excreted in 5 ml urine increased to 414 μU. When 100 μg (2 μl) GABA, which did not itself affect urine flow, was injected also i.vent. 2 min before a second dose of 40 ng carbachol, the mean rate of flow was 140 μl/min and the amount of hormone excreted was 34 μU. A third injection of carbachol alone produced a mean flow of 66 μl/min with the excretion of 245 μU vasopressin. Similar results were obtained in five other experiments. The dose of carbachol required to produce an antidiuretic response by injection into the cisterna magna (i.cist) was 50–100 times that by i.vent. injection in the same rat; i.v. doses up to 1 μg were ineffective. GABA in doses up to 320 μg i.cist. and 1 mg i.v. did not inhibit the antidiuretic response to 5 ng carbachol i.vent. although this response was blocked by 5 μg GABA i.vent. in the same experiments. These results indicate that both carbachol and GABA act on a structure reached from the ventricles, most likely the paraventricular nucleus (PVN). The receptor for carbachol is muscarinic as its effect is blocked by atropine.

GABA has been shown to block the release of vasopressin in response to carotid occlusion by an action at synapses at or near the ventral surface of the medulla (Feldberg & Rocha e Silva, 1979) and the release of milk-ejecting activity from nerve endings in the isolated neural lobe (Dyball & Shaw, 1979). Our experiments suggest an additional inhibitory action on the neurosecretory cell bodies within the PVN. This could possibly mediate recurrent inhibition (Kelly & Renaud, 1978) and is consistent with the finding that micro-iontophoretic application of GABA to these cell bodies in the rabbit causes neuronal inhibition (Moss, Urban & Cross, 1972).

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